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From: Davis, Minh-Tam  
Sent: Friday, September 01, 2006 11:51 AM  
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Subject: FW: Reprint request for 10/077435

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1) Michna, H, 1989, J Steroid Biochem Mol Biol, 34: 447-453.  
2) Schneider MR, 1990, J Steroid Biochem Mol Biol, 1990, 37: 783-787

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From: Davis, Minh-Tam  
Sent: Friday, September 01, 2006 10:59 AM  
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2) El Etreby MF, 1998, Breast cancer res treat, 51: 149-168.  
3) El Etreby MF, 1998, Breast cancer res Treat, 49: 109-117.  
4) Parczky, K, 1996, J Cancer Res Clin Oncol, 122: 283-396.

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## THE ANTITUMOR MECHANISM OF PROGESTERONE ANTAGONISTS IS A RECEPTOR MEDIATED ANTIPROLIFERATIVE EFFECT BY INDUCTION OF TERMINAL CELL DEATH

H. MICHNA,\* M. R. SCHNEIDER, Y. NISHINO  
and M. F. EL ETREBY

Research Laboratories of Schering AG, Müllerstrasse 170-178, D-1000 Berlin 65, F.R.G.

**Summary**—The antiprogestones Onapristone, ZK 112.993 (Schering AG), and Mifepristone (Roussel-Uclaf) proved to possess progesterone receptor-mediated antiproliferative effects in experimental mammary carcinomas. In this study, the potency and mechanism of the antitumor action of Onapristone and ZK 112.993 is characterized by ovariectomized, progestagen and/or estradiol substituted mice bearing hormone-dependent MXT(+) mammary tumours. Medroxyprogesterone acetate (MPA, 0.8 mg/mouse, 3 times weekly, s.c.) could only induce a poor stimulation of tumour growth (% T/C = 40; intact control % T/C = 100), which was only marginally inhibited (% T/C = 21) by Onapristone (0.2 mg/mouse, 6 times weekly, s.c.) during a 6-week therapy. Therefore, the antitumor mechanism of antiprogestones cannot preferably depend on a classical progesterone antagonism. In contrary, the pronounced stimulation of tumor growth (% T/C = 152) by estradiol benzoate (EB, 0.33 µg/mouse, 3 times weekly, s.c.) was completely inhibited (% T/C = 7) by the antiprogestones. An even more stimulated tumour growth was achieved by a combination of EB and MPA (% T/C = 365 using 0.17 mg; % T/C = 225 using 0.8 mg MPA). Onapristone dramatically blocked tumor growth (% T/C = 7) at the lower dose of MPA; no inhibition (% T/C = 203), however, was detected at the higher dose of MPA. These data and a morphological analysis indicate that the potent antitumor activity of the progesterone antagonists depends on the binding to a number of available progesterone receptors high enough to trigger an antiproliferative effect via the induction of terminal differentiation associated with terminal cell death.

### INTRODUCTION

The antiprogestones Onapristone (=ZK 98.299, Schering AG [1-3]) and Mifepristone (Roussel-Uclaf [4]) proved to possess very potent progesterone receptor-mediated antiproliferative effects in several experimental mammary carcinomas (Fig. 1) [5-7]. Our results further indicate that the main mechanism of antitumour action of antiprogestones in these models is a direct progesterone receptor-mediated antiproliferative effect at the level of the mammary tumor cells most probably via the induction of terminal differentiation associated with terminal cell death [8]. Our electron microscopic analysis suggests that it is of pivotal importance for the process of terminal differentiation that the polygonal cells undergo a transformation to the functionally active, mature and nonproliferating secreting cells which are aggregated to acini. This concept is also based on the knowledge that there is an inverse relationship between growth and differentiation and that malignant

tumor cells are considered to have lost their capacity to differentiate terminally; thus, terminal differentiation is the physiological mechanism to stop cell proliferation [9, 10]. There is some evidence from other experimental models that this mammary tumor-inhibiting mechanism of progesterone antagonists is mediated by the progesterone receptor: (1) Antitumor activity is not found in various progesterone receptor-negative cell lines, but is related to progesterone receptor concentrations [11, 12], since after pretreatment of MCF-7 cells with estradiol an increased content of progesterone receptors accompanied by a greater inhibition by RU 486 was detected [12]. (2) Antitumor effects are prevented by the occupation of the progesterone receptor with a progestin [11]. (3) The drug concentration needed to induce antitumor activity *in vitro* is similar to the concentration required to occupy the progesterone receptors [11]. (4) Mifepristone blocks the production of progestin-regulated proteins (receptor mediated) in breast cancer cells [11-15].

To further elucidate the mechanism and potency of the antitumor action of progesterone antagonists in this study, their effects are characterized in ovariectomized, progestagen, and/or estradiol substituted mice.

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## EXPERIMENTAL

The MXT-tumor used was the MXT line M 3.2 originally provided by Dr A. E. Bogden. Two tumor pieces were implanted subcutaneously in female BDF-1 mice. Treatment was started after tumor implantation and randomization on day 1 ( $n = 10-13$ ) for a duration of 31 or 41 days [6]. Ovariectomy was performed on day 1 after tumor implantation. Ovariectomized animals were hormonally substituted with injections of estradiol benzoate (EB) and/or medroxyprogesterone acetate (MPA) subcutaneously 3 times a week in doses given below and already described before [16, 17]. Progesterone antagonists Onapristone, ZK 112.993 (Schering AG), and RU 38.486 (Roussel-Uclaf, Fig. 1) were injected 6 times a week (s.c.) in a dose of 0.2 mg/animal ( $\sim 10$  mg/kg). Tumor growth was determined weekly by caliper measurements. At the end of treatment, tumors and uteri were prepared, weighed, and embedded for histological examinations.

## RESULTS AND DISCUSSION

*Antitumor activity of the antiprogestones in substitution experiments with MPA*

Substitution with MPA only induced a poor stimulation of tumor growth in ovariectomized mice bearing MXT(+) mammary tumors in all doses tested: MPA administration does not stimulate tumor growth comparable to that seen in the intact control animals (Fig. 2). Nevertheless, a dose-dependent growth response to substitution with MPA is evident. Because of this only weak stimulus of MPA on tumor growth (Fig. 2) [16, 17] the potent antitumor activity of antiprogestones [6] cannot preferably depend on a classical, progesterone antagonism. The poor stimulated tumor growth ( $\% T/C = 40$ ; intact control  $\% T/C = 100$ ) after treatment with 0.8 mg/mouse of MPA was only marginally inhibited ( $\% T/C = 21$ ) by Onapristone (0.2 mg/mouse, 5 times weekly, s.c.); Fig. 3).

*Antitumor activity of the antiprogestones in substitution experiments with EB*

In contrast to substitution with MPA, after EB administration (0.33  $\mu$ g/mouse, 3 times weekly, s.c.) the tumor growth attained was identical to that in the

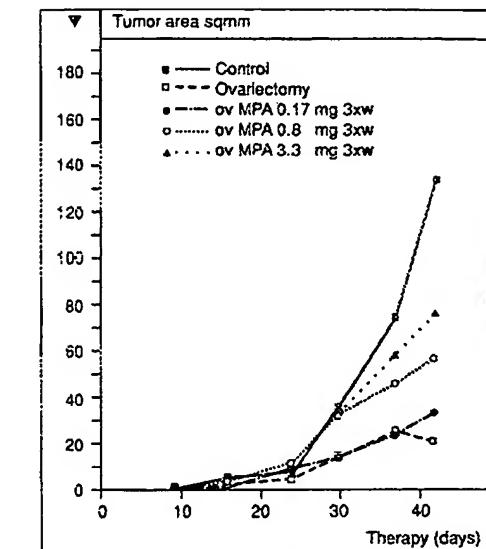


Fig. 2. Growth of hormone-dependent MXT mammary tumors of the mouse in intact (control) or ovariectomized animals substituted with MPA in different doses 3 times a week ( $= 3 \times w$ ).

intact control (Fig. 4). This stimulation was completely inhibited ( $\% T/C = 7$ ) by the antiprogestones Onapristone and ZK 112.993 [Fig. 5(a)]. Due to these data and the fact that the progesterone receptor content in the MXT tumors is enhanced after estrogen treatment [16, 18], the strong antitumor activity of these antiprogestins can be explained by a progesterone receptor-dependent mechanism. In these experiments the weights of uteri revealed no remarkable changes after treatment with the progesterone antagonists in EB substituted animals [Fig. 5(b)]. In contrary, the histological analysis revealed a blocking of estrogenic effects in the endometrial stroma (less edema and less fibroblast activation) compared to the EB substituted control uterus [Fig. 5(c)]. Moreover, multilayered (stratified) formations of the surface and glandular epithelium, necrobiotic changes (apoptotic cell death) in the epithelial lining and the secretory stimulation of glandular structures were evident.

Keeping in mind that mammary carcinomas in general prove to develop out of dysplastic epithelial ducts, it is of great interest that these progesterone

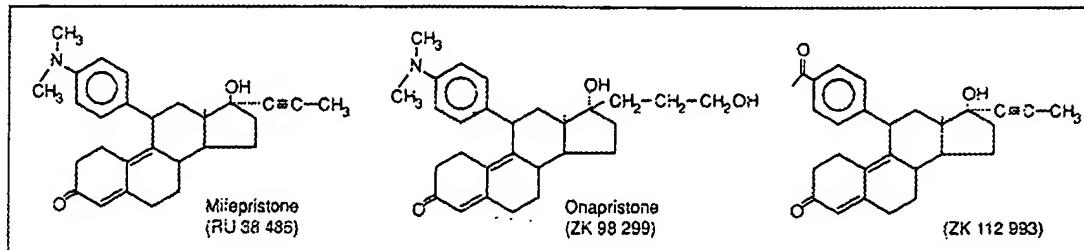


Fig. 1. Chemical structure of Onapristone (Schering AG), ZK 112.993 (Schering AG) and Mifepristone (Roussel-Uclaf).

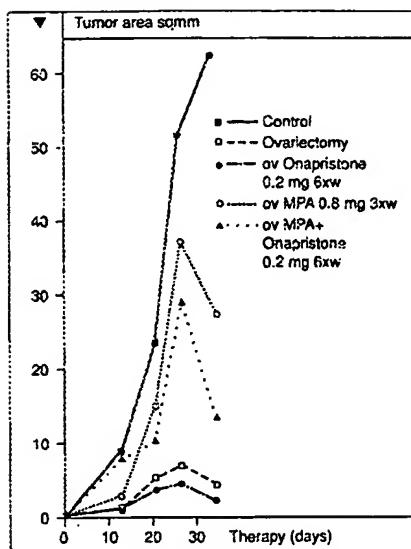


Fig. 3. MXT-tumor growth in intact control or ovariectomized animals substituted with MPA and simultaneous treatment with Onapristone.

antagonists induce massive necrobiosis as well as apoptotic cell death of the epithelial cells of the endometrium and of the uterine glands [Fig. 5(c)].

Interestingly, the blocked tumor growth after treatment with the anti-progestorones in EB substituted mice correlates well with the development of glandular elements and acini displaying secretory activity.

#### Antitumor activity of the antiprogestorones in substitution experiments with EB and MPA

The combination of these hormones in ovariectomized mice caused an enhancement of tumor growth (% T/C = 356) slightly greater than the additive effect of either hormone alone (Fig. 6) [16]

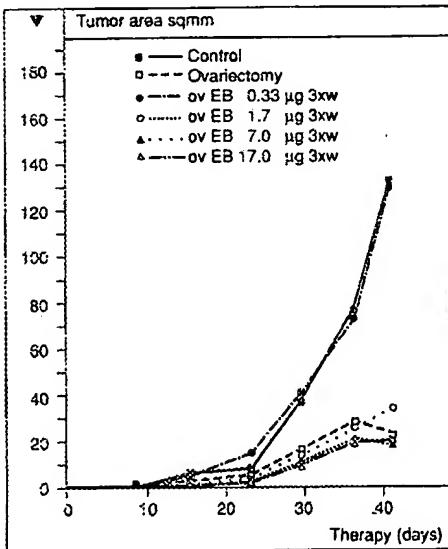


Fig. 4. MXT-tumor growth in intact control or ovariectomized animals substituted with EB.

and the tumor area attained is greater than that of the intact controls. In this combination of EB (0.33 µg/mouse) and MPA (0.17 mg/mouse) and MPA (0.17 mg/mouse) Onapristone completely blocked this strongly enhanced tumor growth (% T/C = 7; Fig. 7). However, by use of a weekly (5-fold higher dose of MPA, Onapristone in the same dose was inactive in inhibiting (% T/C = 203) tumor growth (Fig. 8).

These two experiments indicate that the potent antitumor activity of the progesterone antagonists depends on the availability of a sufficient number of free (unoccupied) progesterone receptors, since the antitumor activity disappeared when the progesterone receptors are blocked or occupied by an increased number of MPA molecules.

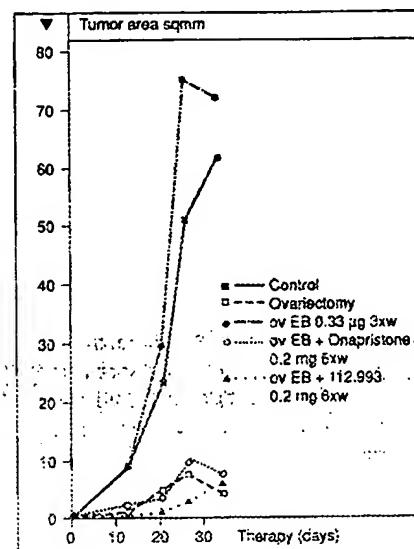


Fig. 5(a)

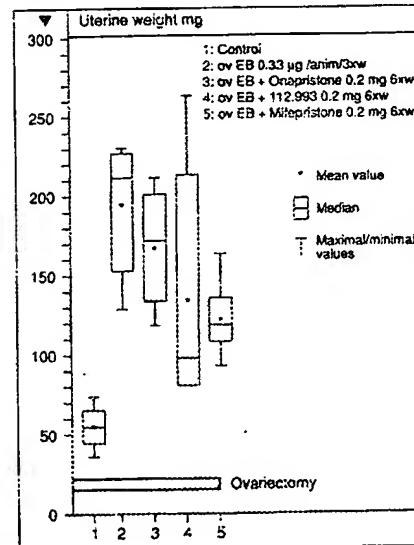


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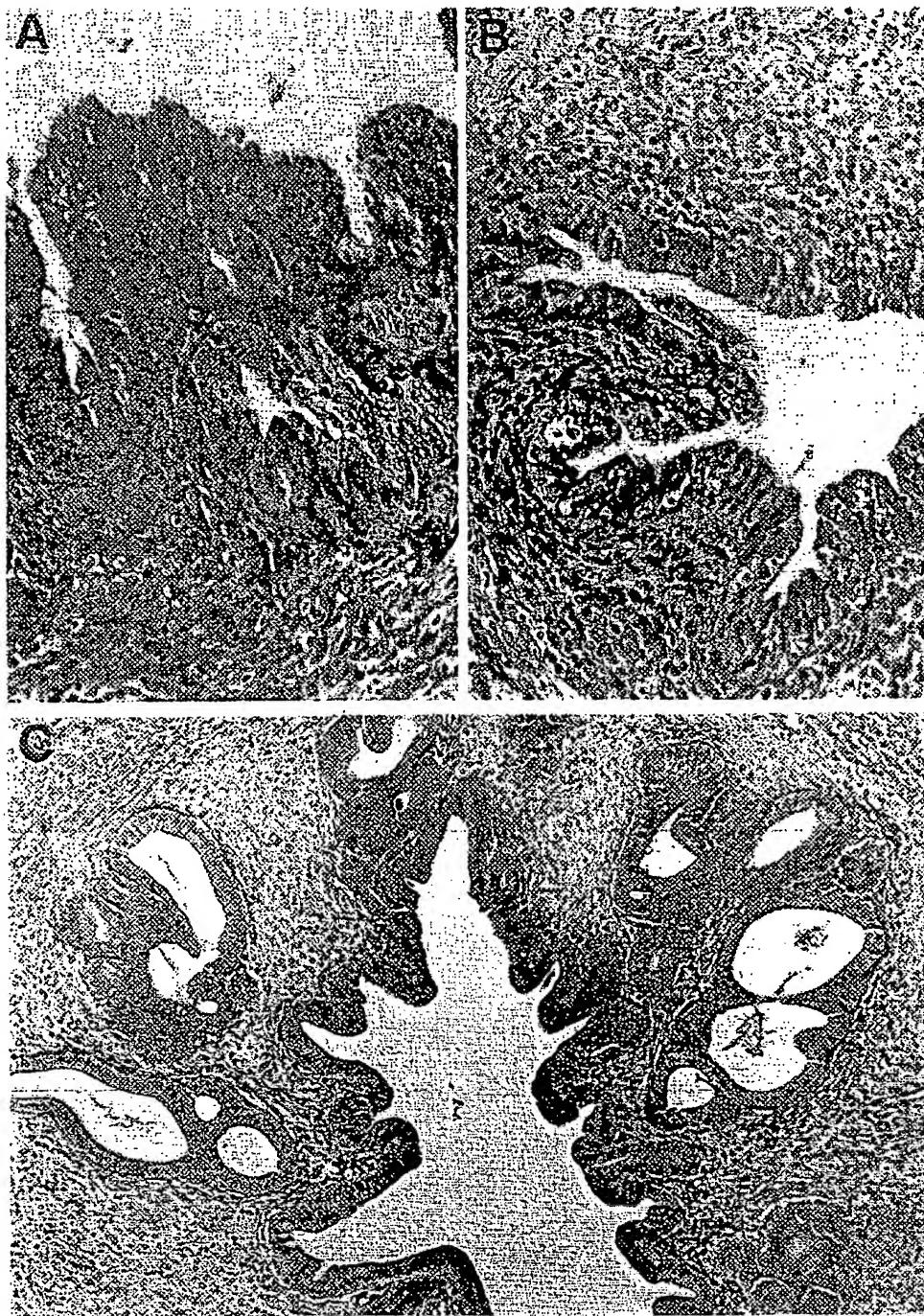


Fig. 5(c)

Fig. 5. (a) MXT-tumor growth pattern after substitution with EB and simultaneous treatment with progesterone antagonists. (b) Wet weights of uteri in ovariectomized mice, substituted with EB alone or after simultaneous treatment with different progesterone antagonists. (c) Morphological reaction pattern of the uterine epithelium after ovariectomy and substitution of EB (C) or simultaneous treatment with Onapristone (A) or ZK 112.993 (B). Note differentiation of the epithelium with massive and specific induction of necrobiosis and apoptotic cell death (= antiepithelial effect) after treatment with progesterone antagonists.

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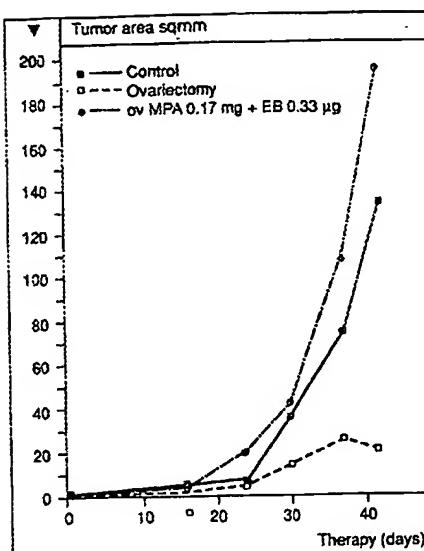


Fig. 6. MXT-tumor growth pattern after substitution of ovariectomized mice with MPA (0.17 mg) and EB (0.33 µg).

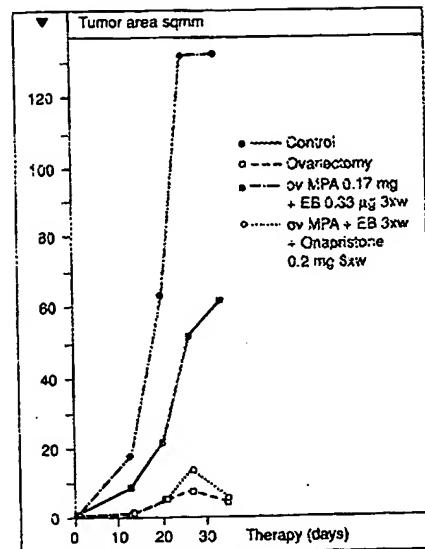


Fig. 7. Tumor growth after substitution with MPA (0.17 mg) and EB (0.33 µg) as well as additional treatment with Onapristone (0.2 mg).

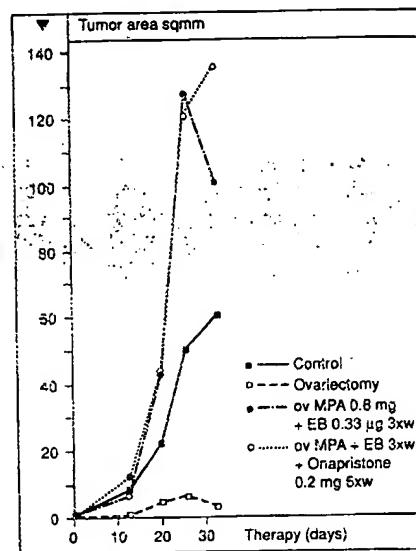


Fig. 8. MXT-tumor growth pattern after substitution with MPA (0.8 mg) and EB (0.33 µg) as well as additional treatment with Onapristone (0.3 mg).

Tumor inhibition by the antiprogestones was found again to correlate with a pronounced appearance of glandular structures and acini, as well as the induction of secretory activity in these mammary tumors. Furthermore, tumor areas displaying no differentiation to glandular structures and acini were characterized by an enhanced amount of the spindle-shaped necrobiotic cell population. The morphological analysis establish the concept that progesterone antagonists, like Onapristone and ZK 112.993, trigger an antiproliferative effect in these mammary carcinomas by the induction of differentiation of the mitotically active polygonal tumor cells towards secretory active glandular structures, as well as

towards spindle-shaped resting and necrobiotic subpopulations (Fig. 9).

It appears that these antiprogestins possess the potency to eliminate an intrinsic block in terminal differentiation in progesterone receptor positive mammary tumor cells of the mouse and the rat. In addition, it is to consider whether this mechanism is also responsible for the induction of necrobiosis in uterine epithelial cells. The alterations of the endometrium and the necrobiosis of the uterine epithelial cells stimulate the idea to consider this concept of terminal differentiation and terminal cell death in explaining the mechanism of action of progesterone antagonists in a therapy of endometriosis.

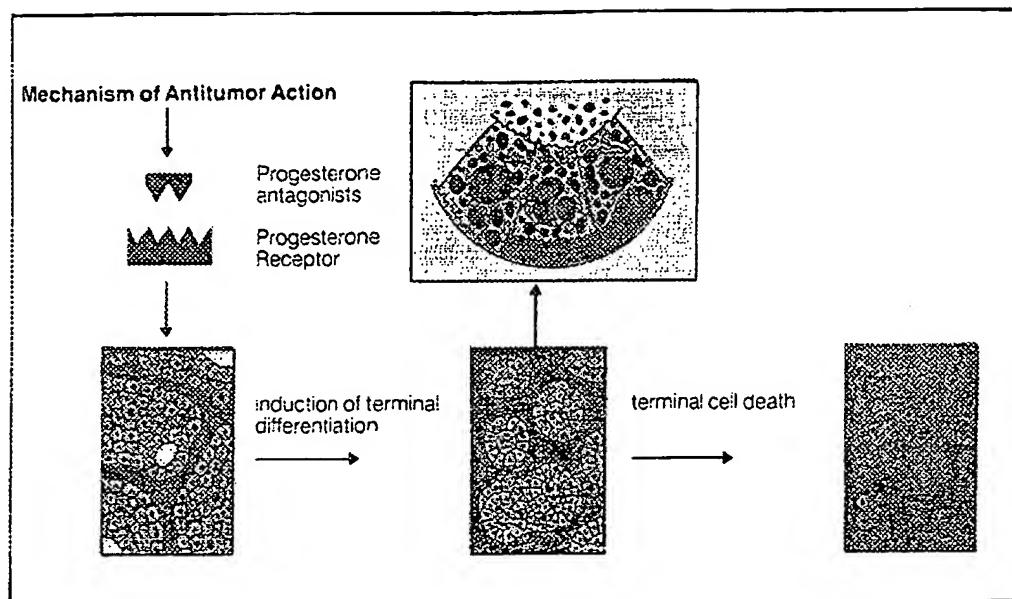


Fig. 9. Concept of terminal differentiation as an explanation for the tumor-inhibiting mechanism of progesterone antagonists in experimental mammary adenocarcinomas based on light and ultrastructural investigations.

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These data with MXT(+) tumors, as well as the morphological analysis, favor the concept that the potent antitumor activity of the progesterone antagonists Onapristone and ZK 112.993 (Schering) depends on the binding to a sufficient number of available progesterone receptors high enough to trigger an proliferative effect by induction of terminal differentiation leading to terminal cell death.

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## ***9 Differentiation and Apoptosis as a Therapeutic Strategy for Hormone-Dependent Cancers***

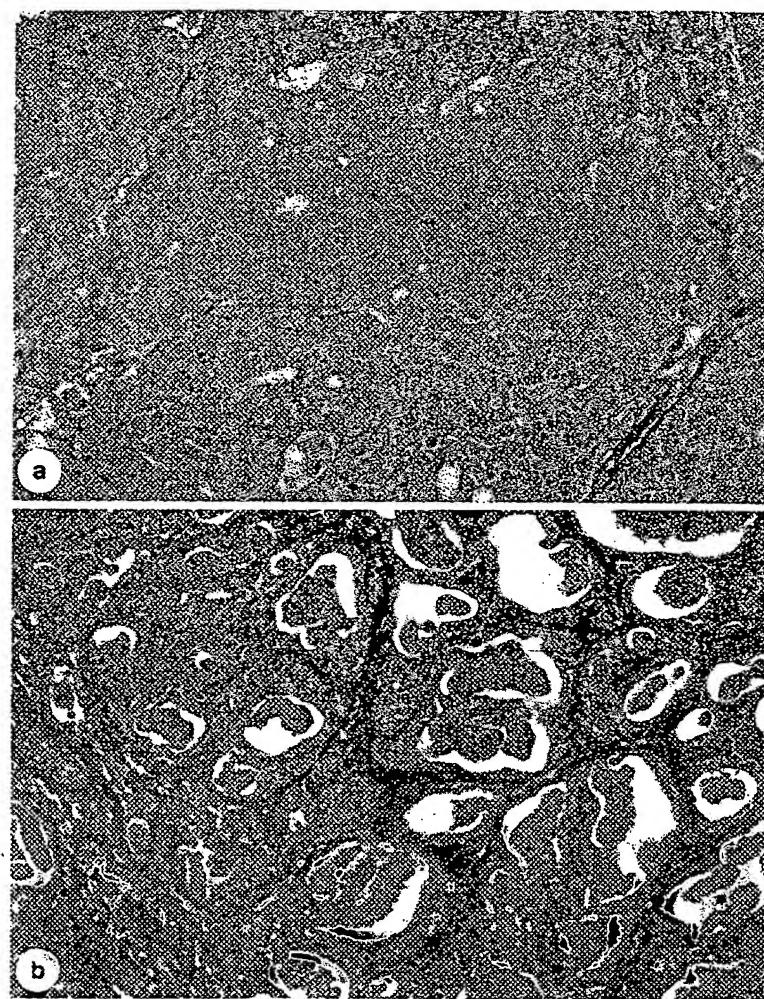
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H. Michna and K. Parczyk

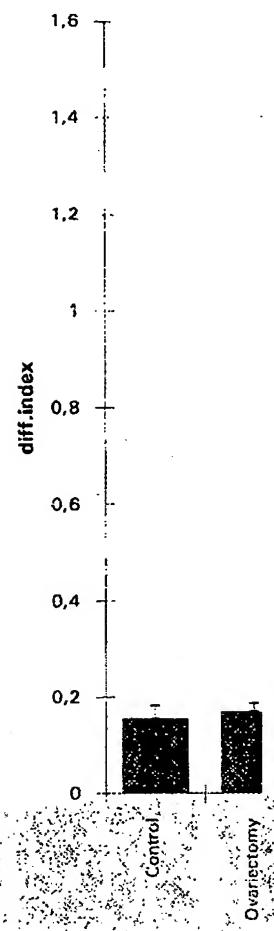
Our research efforts have been aimed at identifying a compound for the treatment of hormone-dependent cancers. For this purpose the progesterone antagonist onapristone has been selected (Neef et al. 1983; Michna et al. 1989a; Schneider et al. 1989) and is now in a phase III clinical trial. The ability of this compound class to prevent the growth of mammary carcinomas requires the presence of progesterone receptors within the tumors (Michna et al. 1989b). These compounds neither block the secretion of pituitary or ovarian hormones nor are they cytotoxic drugs in experimental rodent models. In addition, the tumor inhibitory potential of progesterone antagonists has proven to be independent of their antihormonal (= antiprogestational) activity and these compounds exert their tumor-inhibiting potential even in the absence of progesterone (Michna et al. 1989b).

Considering the physiological function of the progesterone receptor, which is the mediation of differentiation, it seemed expedient to analyze the morphology of mammary carcinomas after treatment with progesterone antagonists. From comparative studies in MXT, DMBA, and NMU breast cancer models we draw the following conclusions:

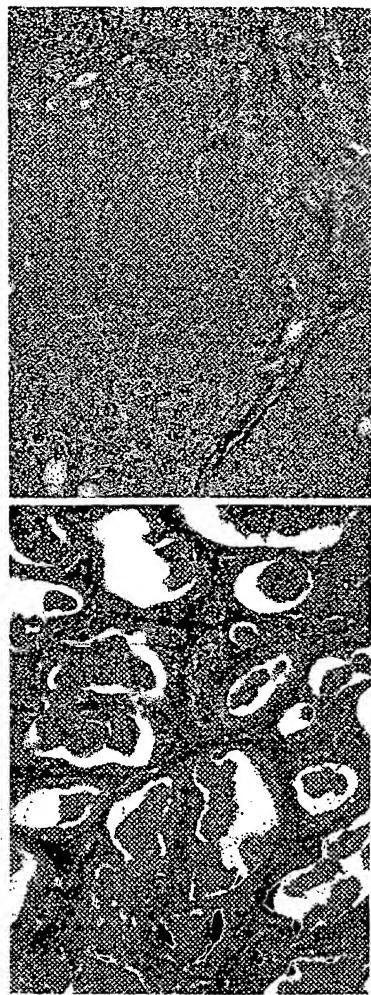
In experimental breast cancer models we detected that the tumor inhibition of progesterone antagonists, such as onapristone was accompanied by a shift of undifferentiated tumor epithelial cells towards glandular structures.



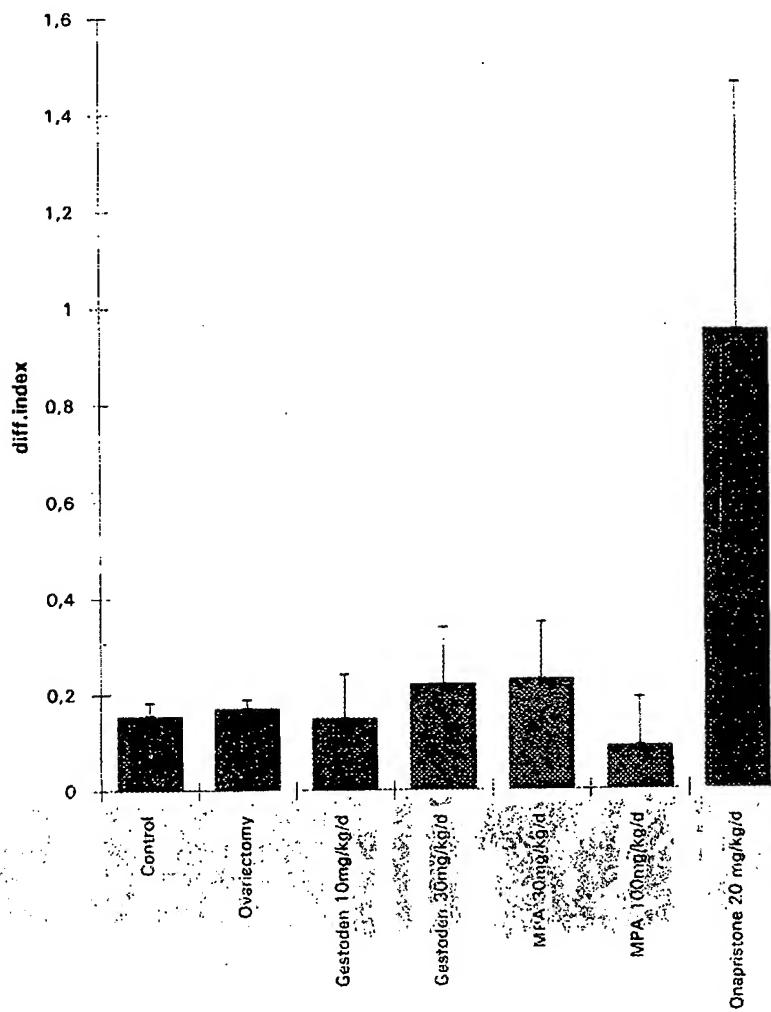
**Fig. 1a,b.** DMBA-induced mammary carcinoma of the rat; light microscopic characteristics of untreated control and progesterone antagonist (onapristone)-treated tumors after azane staining. It is obvious that after treatment with onapristone more dysplastic glandular structures are filled with secretory material,  $\times 180$



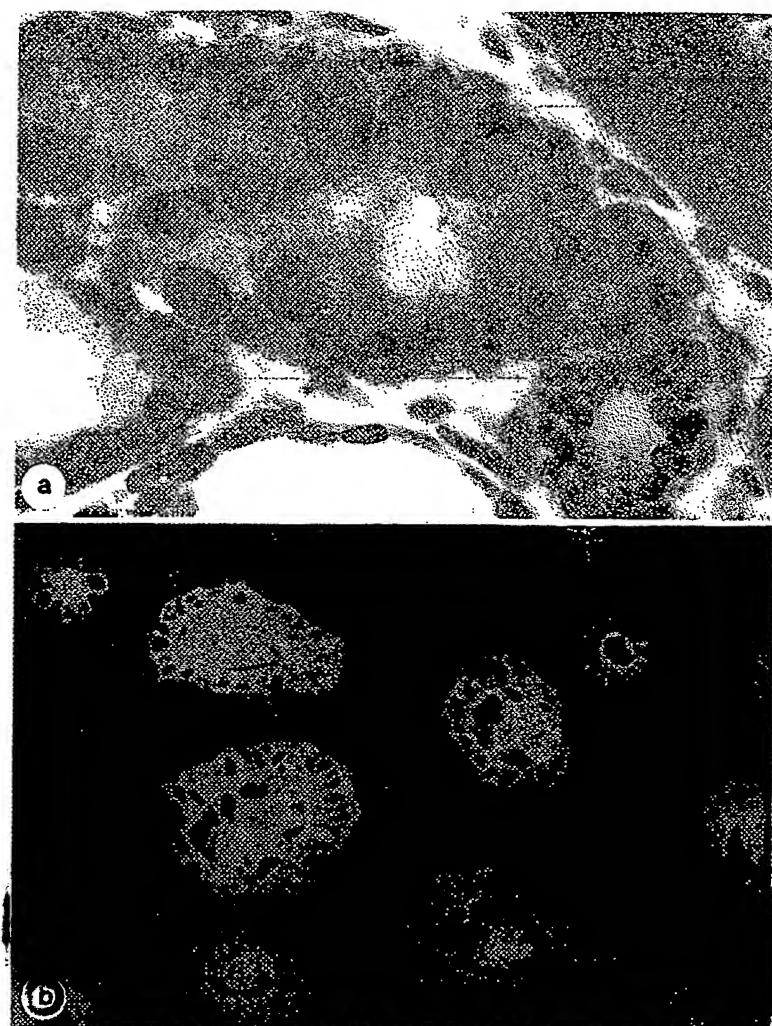
**Fig. 2.** Using morphome structures and undifferentiated in DMBA-induced differentiation index"; no effect of ovariectomy, whereas the differentiation index



carcinoma of the rat; light microscopic progestosterone antagonist (onapristone)-obvious that after treatment with onapristone are filled with secretory material,



**Fig. 2.** Using morphometrical procedures the volume density of glandular structures and undifferentiated, spindle-shaped tumor epithelial cells was estimated in DMBA-induced mammary carcinomas and used to calculate a "differentiation index"; no effect was seen after treatment with high doses of progestins, such as gestoden and medroxyprogesteroneacetate (MPA), or after ovariectomy, whereas treatment with an antiprogestin significantly enhanced the differentiation index



**Fig. 3.** a Staining of progesterone antagonist (onapristone) treated-DMBA induced mammary carcinomas with "oil-red" revealed the secretion of fatty acids; the secreted material is stained in red,  $\times 920$ . b In addition, fluorescence immunohistochemistry detected the secretion of casein,  $\times 320$

This reaction pattern v light microscopy (Fig. 1), morphometrical procedur estimating and bringing spindle-shaped cells to di dysplastic, ductlike struct differentiation of hormo: NMU, and DMBA tum progesterone antagonists, ifen, or high doses of e: degree of differentiation ( using classical grading sy: system (Gehring et al. 19 that only after treatment epithelial cells show clea carcinoma cells secrete i addition to secretion, tra: totic tumor epithelial cell: and Merker 1973; Bursch biotic tumor cells domina

The estimation of indi: tions revealed that all en such as ovariectomy, trea: antiestrogen ICI 164 38: with high doses of estro: apoptosis; the most sign: treatment with an anti: ferences were noted in m: with recent reports indi: apoptotic cell death in m: non and Rochefort 1994)

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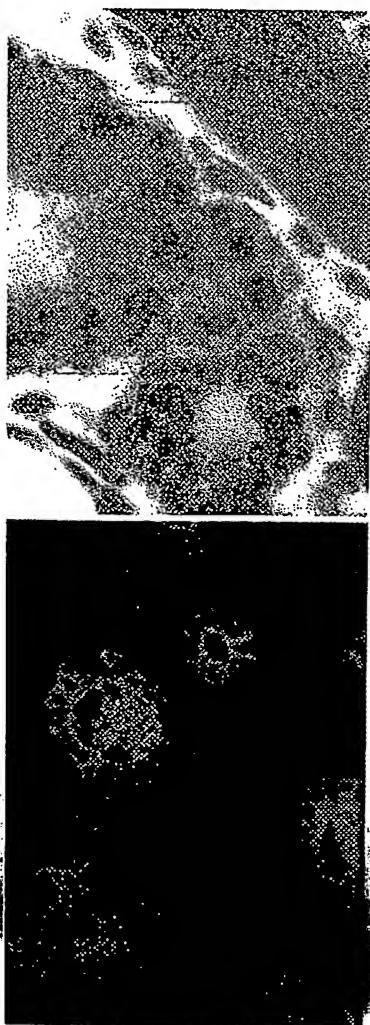
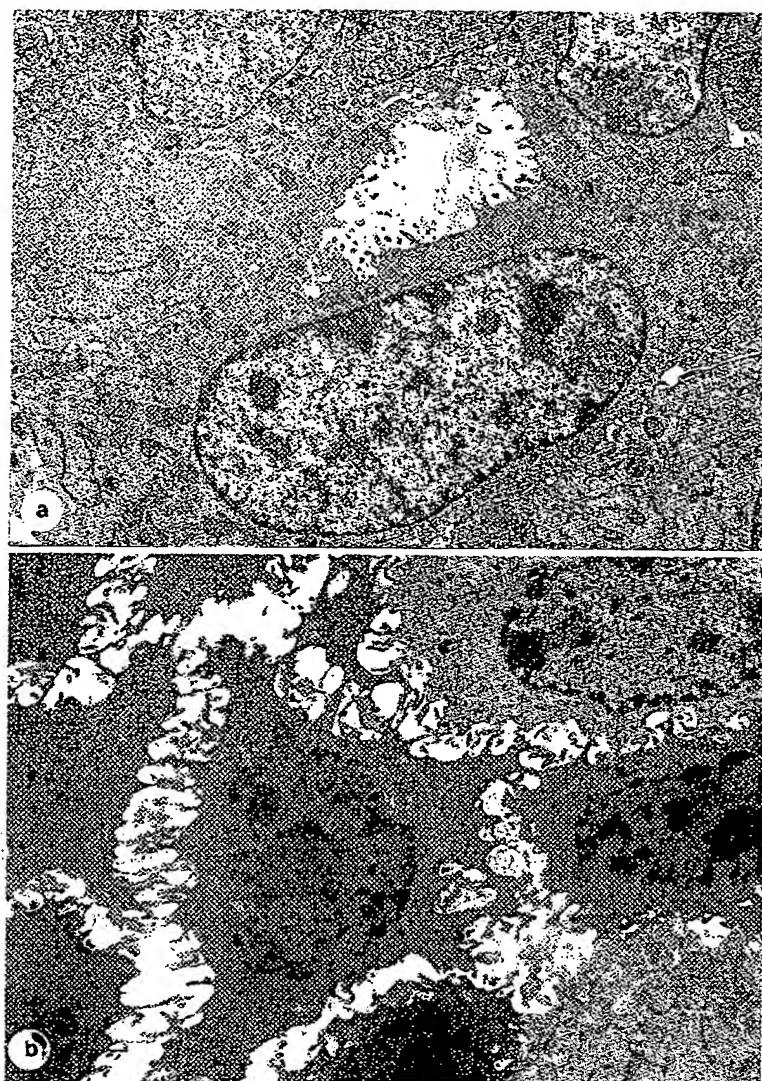


Fig. 1. a In a non-tumorigenic (onapristone) treated-DMBA induced tumor revealed the secretion of fatty acids (red,  $\times 920$ ). b In addition, fluorescence microscopy revealed the secretion of casein (red,  $\times 320$ ).

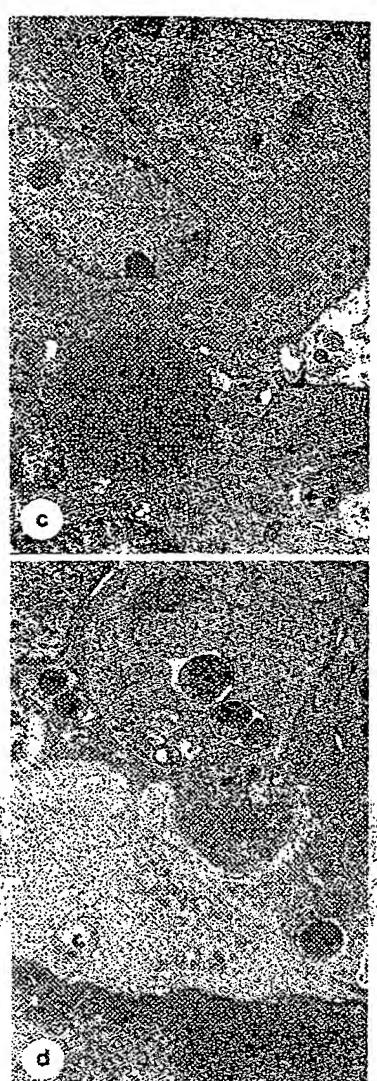
This reaction pattern was already obvious at the qualitative level of light microscopy (Fig. 1). We could also ensure this reaction by using morphometrical procedures introducing a new differentiation factor, estimating and bringing into relation the amount of undifferentiated, spindle-shaped cells to differentiated tumor epithelial cells arranged to dysplastic, ductlike structures (Gehring et al. 1991). A higher degree of differentiation of hormone-dependent mammary carcinomas (MXT, NMU, and DMBA tumors) was only detected after treatment with progesterone antagonists, whereas ovariectomy, treatment with tamoxifen, or high doses of estrogens or progestins did not influence the degree of differentiation (Fig. 2). The same conclusion could be drawn using classical grading systems such as the WHO or Richardson grading system (Gehring et al. 1991). In line with the above, our data indicate that only after treatment with progesterone antagonists did the tumor epithelial cells show clear evidence of secretory activation – mammary carcinoma cells secrete fatty acids (Fig. 3a) and casein (Fig. 3b). In addition to secretion, transmission electron microscopy revealed apoptotic tumor epithelial cells in breast tissue (Kerr et al. 1972; Schweichel and Merker 1973; Bursch et al. 1985), whereas after ovariectomy necrotic tumor cells dominate the picture (Fig. 4).

The estimation of indices of mitotic and apoptotic tumor cell reactions revealed that all endocrine treatment strategies of breast cancer, such as ovariectomy, treatment with tamoxifen, treatment with the pure antiestrogen ICI 164 384 (Wakeling and Bowler 1987), or treatment with high doses of estrogen or progestins may enhance the degree of apoptosis; the most significant apoptotic reactions were observed after treatment with an antiprogestin (Fig. 5a), whereas no significant differences were noted in mitogenic reactions. These data are in agreement with recent reports indicating that tamoxifen derivatives may induce apoptotic cell death in mammary cancer models (see overview in Vignon and Rochefort 1994).

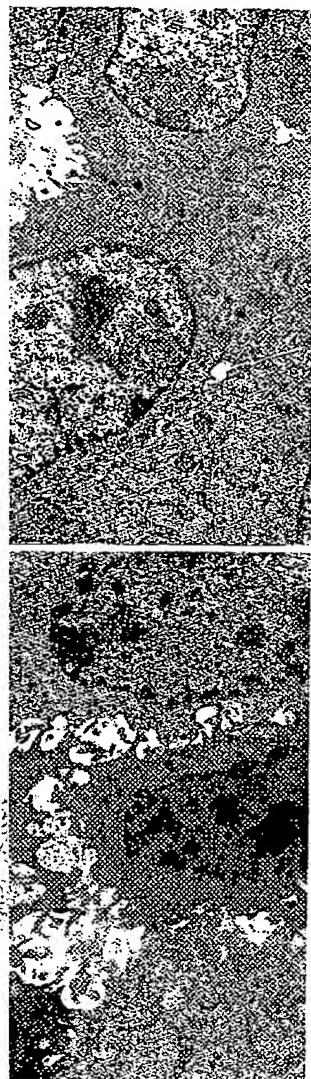
Since it was also shown earlier that tamoxifen may stimulate the secretion of the "negative" growth factor transforming growth factor- $\beta_1$  (TGF- $\beta_1$ ) in breast cancer tissue of patients treated with tamoxifen (Colletta et al. 1992) and that (liver) cells undergoing active cell death by apoptosis secrete TGF- $\beta_1$  (Bursch et al. 1993), we studied the expression of TGF- $\beta_{1,2,3}$  within the experimental mammary carcinomas at the mRNA level and protein level. It was only at the protein level that



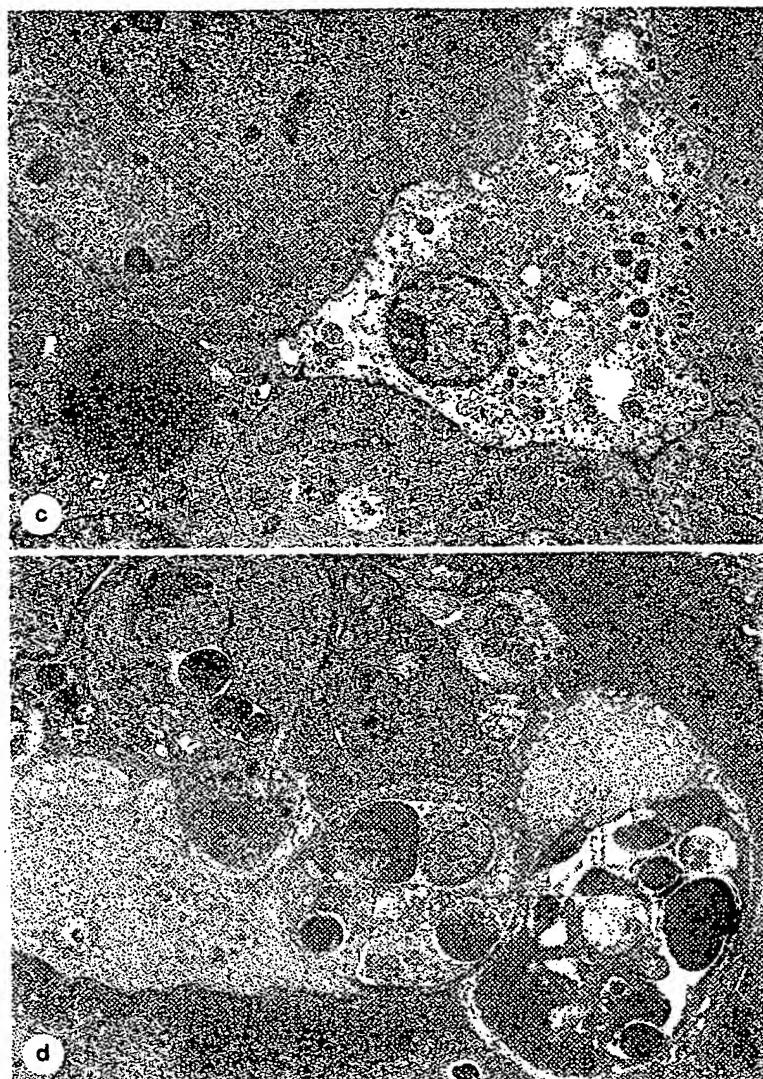
**Fig. 4a-d.** Transmission electron microscopy of MXT mammary carcinomas of the mouse. Whereas after ovariectomy the tumors display necrobiotic reactions (b), in comparison to control tumors grown on intact animals (a) after treatment with an antiprogestins, the tumor cells are arranged to glandular



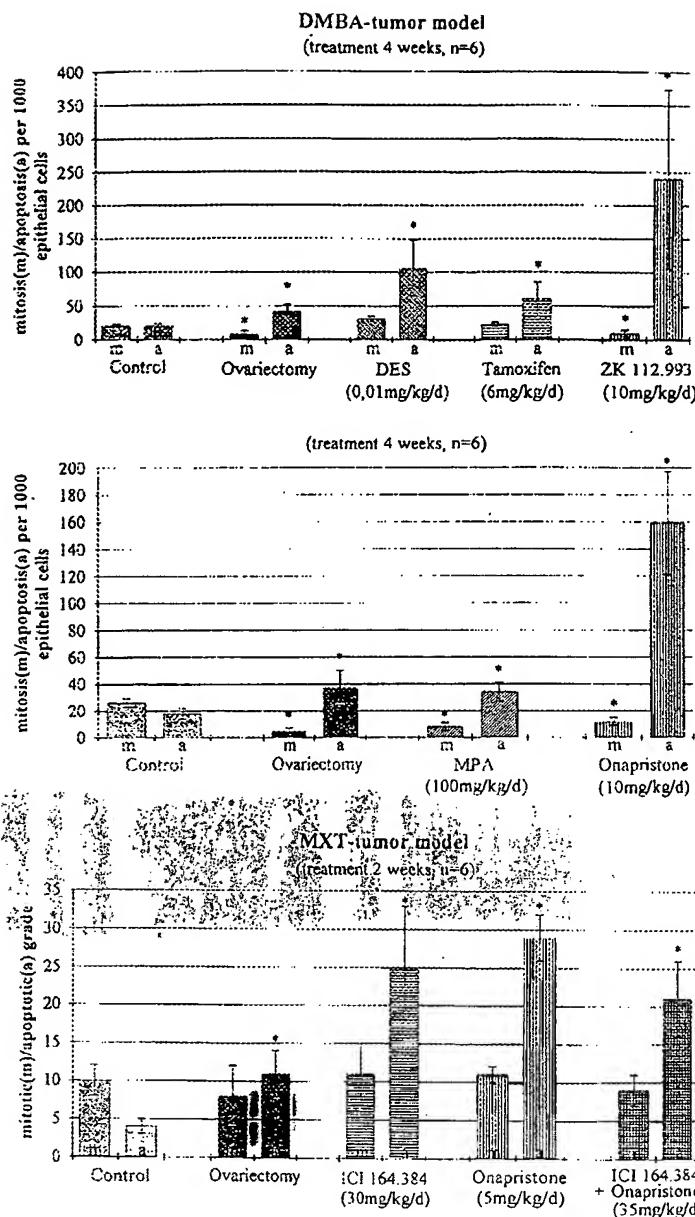
structures bearing signs of secretory :  
apoptotic cell death (d) expressing :  
rochromatine dense material,  $\times 9600$



of MXT mammary carcinomas tumors display necrobiotic reaction on intact animals (a) after cells are arranged to glandular



structures bearing signs of secretory activity (c). They contain cells undergoing apoptotic cell death (d) expressing the characteristic apoptotic nuclear, heterochromatin dense material,  $\times 9600$



## Differentiation and Apoptosis

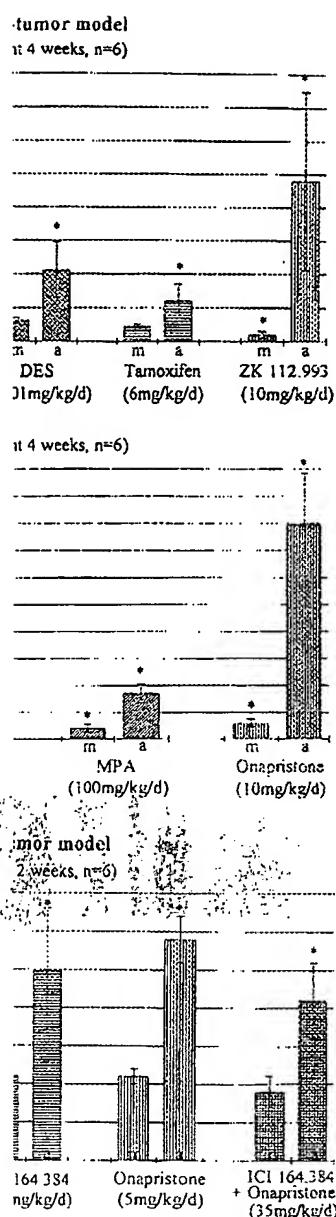
**Table 1. TGF  $\beta$ 1: immunhistochemical analysis**

EO 91-0416 MNU tumor model (treatment 4 weeks)	Stromal tissue
Control (n = 9; N = 13)	3.0 ( $\pm$ 0.6)
Ovariectomy (n = 4; N = 4)	1.4 ( $\pm$ 0.5)
Tamoxifen (n = 6; N = 10) (5 mg/kg per day)	3.7* ( $\pm$ 0.7)
Onapristone (n = 13; N = 14) (20 mg/kg per day)	1.6* ** ( $\pm$ 0.8)

\* Significant difference to control; \*\* significant difference to Tamoxifen ( $p < 0.05$ ); m, mitosis; a, apoptosis

we could detect a higher degree of differentiation and a higher degree of apoptosis in the stromal tissue. After 4 weeks of treatment with tamoxifen a higher degree of differentiation was observed in stromal cells, after 4 weeks of treatment with onapristone a higher degree of apoptosis was observed in stromal cells as assessed by semiquantitative morphometric analysis. These data are in agreement with our previous results (Knabbe et al. 1987; Jei-

**Fig. 5. Morphometric analysis of mitosis and apoptosis in the stromal tissue of MNU-induced and MXT mammary carcinomas treated with different hormone therapies; m, mitosis; a, apoptosis. The data show that treatment with the progestrone antagonist onapristone, but not the pure antiestrogen ICI 164.384, influences the degree of differentiation and apoptosis in the stromal tissue. The combination of diethylstilbestrol; MPA, medroxyprogesterone acetate; 2K 112.993, tamoxifen; ICI 164.384, pure antiestrogen; Ovariectomy, ovariectomy; Control, control**

Table 1. TGF  $\beta$ 1: immunohistochemical scoring

EO 91-0416 MNU tumor model (treatment 4 weeks)	Stromal tissue	Epithelial tissue	Glandular lumina
Control (n = 9; N = 13)	3.0 ( $\pm$ 0.6)	2.2 ( $\pm$ 0.9)	2.0 ( $\pm$ 0.6)
Ovariectomy (n = 4; N = 4)	1.4 ( $\pm$ 0.5)	1.8 ( $\pm$ 0.6)	2.5 ( $\pm$ 0.6)
Tamoxifen (n = 6; N = 10) (5 mg/kg per day)	3.7* ( $\pm$ 0.7)	2.6 ( $\pm$ 0.6)	2.1 ( $\pm$ 0.6)
Onapristone (n = 13; N = 14) (20 mg/kg per day)	1.6* ** ( $\pm$ 0.8)	3.5* ** ( $\pm$ 0.8)	2.9* ** ( $\pm$ 0.8)

\* Significant difference to control ( $p < 0.05$ ; KRWA test); \*\* significant difference to Tamoxifen ( $p < 0.05$ ; KRWA test).

we could detect a higher degree of TGF- $\beta$ 1 secretion by immunohistochemical examination after treatment with progesterone antagonists (Fig. 6a,b), and the degree of staining with the proliferation marker PCNA was reduced simultaneously (Fig. 6a,b). Whereas after treatment with tamoxifen a higher degree of TGF- $\beta$ 1 immunostaining was localized in stromal cells, after treatment with progesterone antagonists such as onapristone higher staining was displayed by tumor epithelial cells as assessed by semiquantitative analysis (Table 1). Finally, these data are in agreement with *in vitro* observations, indicating that TGF- $\beta$  is a hormonally regulated negative growth factor in human breast cancer cells (Knabbe et al. 1987; Jeng and Jordan 1991).

◀ Fig. 5. Morphometric analysis of the mitotic and apoptotic reaction in DMBA-induced and MXT mammary carcinomas after blockade of tumor growth with different hormone therapies; most significant apoptotic reactions after treatment with the progesterone antagonists onapristone or ZK 112.993; nevertheless the pure antiestrogen ICI 164.384 and also the other less pronounced hormone regimen may influence apoptosis when given in high doses. DES, diethylstilbestrol; MPA, medroxyprogesteroneacetate

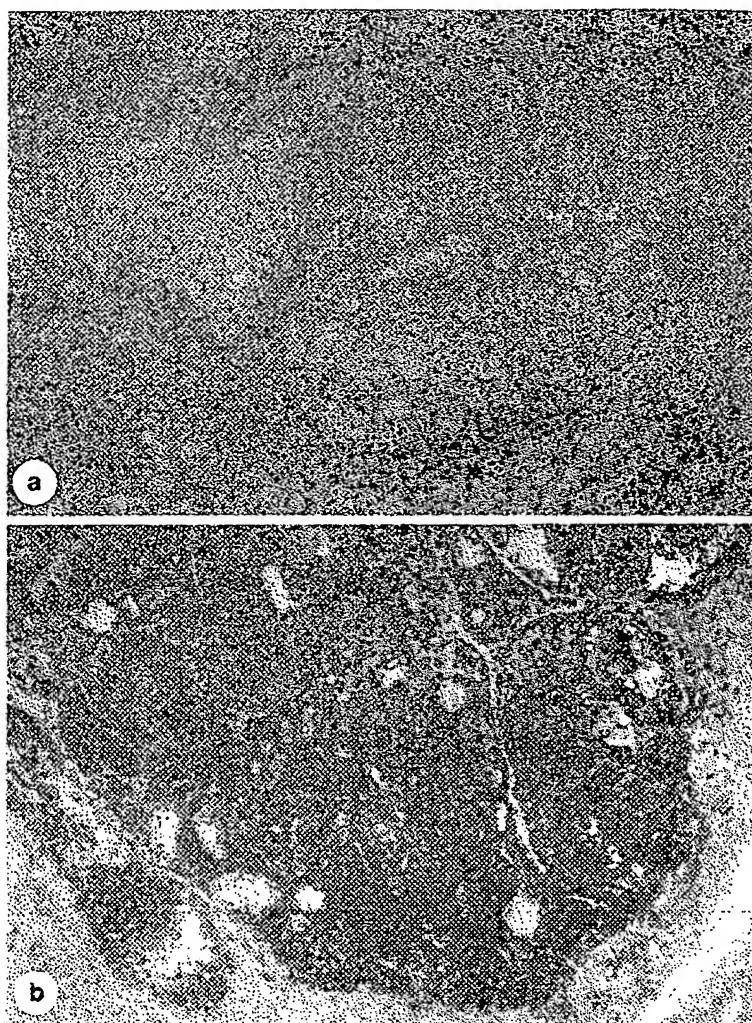


Fig. 6a,b. Coexpression studies by use of immunohistochemistry of the growth factor TGF- $\beta$  and the proliferation marker proliferating cell nuclear antigen (PCNA); reduction of PCNA staining and enhanced epithelial localization of TGF- $\beta$  after treatment of DMBA-induced mammary carcinomas with the antiprogestin onapristone,  $\times 90$

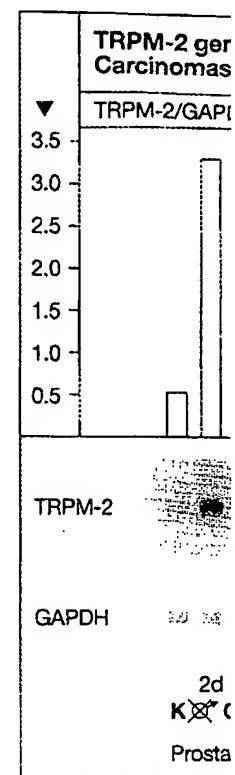
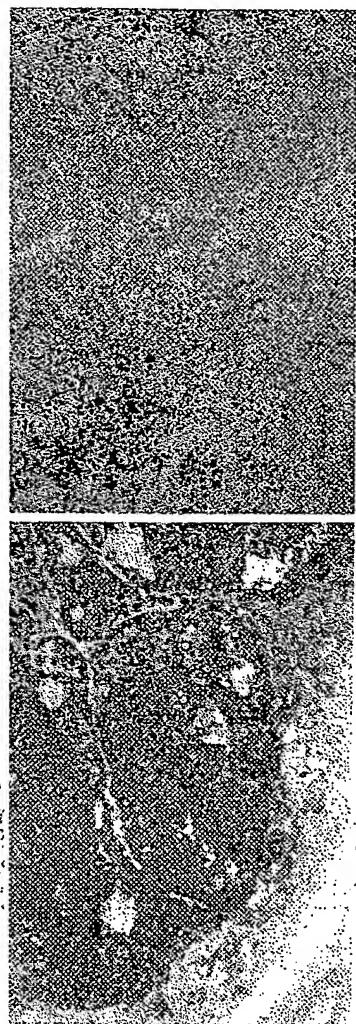
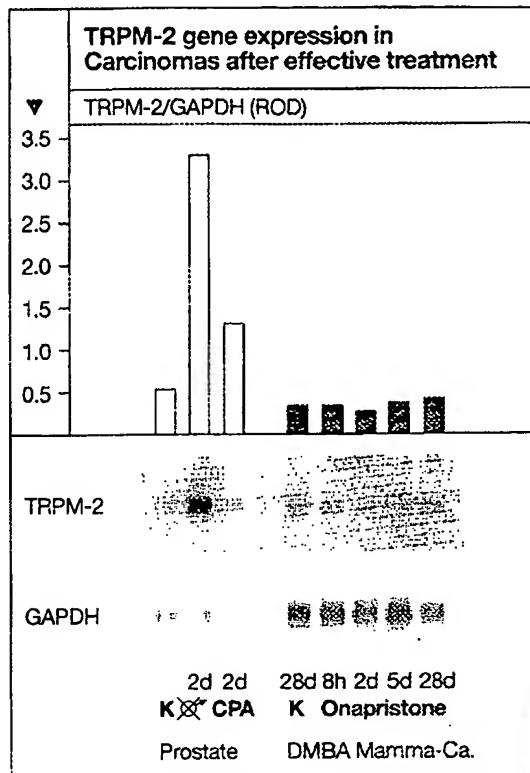


Fig. 7. Northern blot analysis of TRPM-2 expression in ger carcinomas of orchectomy or treatment with 10 mg/kg per day of the rat progestin DMBA-induced mammary carcinoma. The samples were pooled from

Interestingly, observations of hormonal control of cell differentiation somehow linked (Walker et al. has already been proposed a mechanism of the tumor cell accumulation of the hormone-dependent transcriptional antagonists (Michna et al. 1990).

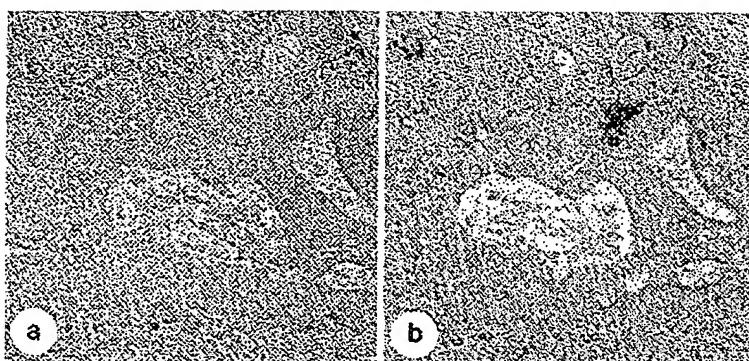


of immunohistochemistry of the marker proliferating cell nuclear and enhanced epithelial localiza- induced mammary carcinomas with



**Fig. 7.** Northern blot analysis of TRPM-2 gene expression in rat tissue. Effects of orchiectomy or treatment with the antiandrogen cyproterone acetate (CPA; 10 mg/kg per day) on the rat prostate. Effects of onapristone (5 mg/kg per day) on DMBA-induced mammary carcinomas after 8 h, 2, 5, and 28 days of treatment. The samples were pooled from five individual prostates/tumors

Interestingly, observations in some stem cell types indicate that hormonal control of cell differentiation and cell cycle changes are somehow linked (Walker et al. 1993), and differentiation-specific arrest has already been proposed as this link. Keeping this in mind, the accumulation of the tumor cells in G<sub>0</sub>/G<sub>1</sub> of the cell cycle after treatment of hormone-dependent breast cancers *in vivo* with progesterone antagonists (Michna et al. 1990) lends further support to the concept that



**Fig. 8a,b.** Immunohistochemical staining of TRPM-2 in DMBA-induced mammary carcinomas after treatment with onapristone. **a** IgG control without the TRPM-2 antibody; note focal expression of TRPM-2,  $\times 180$

the antitumor action of antiprogestins is related to the induction of differentiation leading to active cell death.

It has been shown that the gene TRPM-2 (testosterone repressed prostate message) or clusterin is expressed in a wide variety of tissues undergoing active cell death (Montpetit et al. 1986; Rennie et al. 1988; Tenniswood et al. 1990), especially secretory epithelial cells in the prostate and mammary gland, although the functional significance of TRPM-2 is still under investigation (Wilson et al. 1994). Unlike an enhanced expression of the TRPM-2/clusterin gene, after orchiectomy in regressing normal prostate tissue (Fig. 7) as was originally discovered by Léger et al. (1988), we could not detect an upregulation either by ovariectomy or treatment with progesterone antagonists in the analysis of the mRNA levels in homogenates of regressing DMBA-induced mammary carcinomas (Fig. 7). Nevertheless, the immunohistochemical findings of the expression of the clusterin/TRPM-2 gene have revealed that the focal expression is enhanced in regressing mammary carcinomas after treatment with antiprogestins (Fig. 8).

Apoptotic cells similar to those seen in mammary carcinoma epithelial cells were also detected in progesterone receptor-positive uterine epithelial cells (Michna et al. 1989b). Most interestingly, there was also an increase in apoptotic cell death in the absence of progesterone in

## Differentiation and Apoptosis a

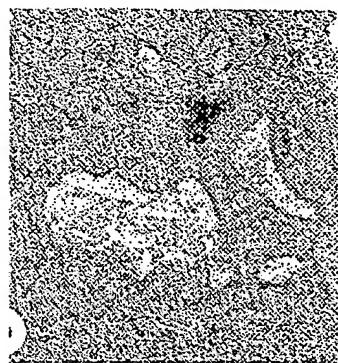
**Table 2.** Morphological reaction bearing mice treated with estradiol different progesterone antagonists

Treatment	In of [%] (n)
OV + EB <sup>a</sup>	15 (n)
OV + EB + ZK 98.299 (10 mg/kg per day)	45 (n)
OV + EB + RU 486 (10 mg/kg per day)	32 (n)

Note enhanced appearance of ep and differentiation of the superfic multirowed and even multilayered EB, estradiol benzoate; OV, ovaria  
<sup>a</sup>Application of compounds all su

ovariectomized estradiol-treat ciated with the expression of t the progesterone antagonists death pathway of epithelial ce or uteri. Nevertheless, our stu have revealed the involveme gression by the mechanism (Ramer et al. 1992). These and o stromal–epithelial interactions ism of active cell death as wel

These data have led us to gonists, such as onapristone, i gesterone receptor and medi plicated in active cell death, fi favor the idea that specific gr epithelial cell death (Kyprian Chung et al. 1992). However,



TRPM-2 in DMBA-induced mammary carcinoma. a IgG control without the TRPM-2,  $\times 180$

is related to the induction of death.

TRPM-2 (testosterone repressed mRNA) is expressed in a wide variety of tissues (Kaufmann et al. 1986; Rennie et al. 1988; secretory epithelial cells in the uterus) and has the functional significance of clusterin (Wilson et al. 1994). Unlike an androgen receptor gene, after orchiectomy (Fig. 7) as was originally discovered for clusterin, upregulation either by androgen antagonists in the analysis of regressing DMBA-induced carcinomas, the immunohistochemical analysis of TRPM-2 gene have revealed that in regressing mammary carcinomas (Fig. 8).

in mammary carcinoma epithelial cells, the receptor-positive uterine carcinomas interestingly, there was also the absence of progesterone in

**Table 2.** Morphological reactions in ovariectomized, mammary carcinoma-bearing mice treated with estradiol benzoate and simultaneously treated with different progesterone antagonists

Treatment	Incidence of apoptotic nuclei [%]	Morphology of epithelium
OV + EB <sup>a</sup>	15 $\pm$ 5 (n = 4)	Single layer
OV + EB + ZK 98.299 (10 mg/kg per day)	42 $\pm$ 6 (n = 4)	Multirowed, multilayered
OV + EB + RU 486 (10 mg/kg per day)	32 $\pm$ 9 (n = 6)	Multirowed, multilayered

Note enhanced appearance of epithelial cells undergoing apoptotic cell death and differentiation of the superficial uterine epithelium from single-layered to multirowed and even multilayered epithelium.

EB, estradiol benzoate; OV, ovariectomized mice.

<sup>a</sup>Application of compounds all subcutaneously.

ovariectomized estradiol-treated animals (Table 2), which is also associated with the expression of the clusterin/TRPM-2 gene (Fig. 9). Thus, the progesterone antagonists specifically interact with the active cell death pathway of epithelial cells without killing stromal cells in tumors or uteri. Nevertheless, our studies on the marker glycoprotein tenascin have revealed the involvement of stromal reactions during tumor regression by the mechanism apoptosis induced by antiprogestins (Vollmer et al. 1992). These and other data (Cunha et al. 1987) suggest that stromal-epithelial interactions appear to be critical both for the mechanism of active cell death as well as cell survival.

These data have led us to propose that progesterone receptor antagonists, such as onapristone, use the physiological function of the progesterone receptor and mediate differentiation and stimulate genes implicated in active cell death, finally leading to apoptosis. These data also favor the idea that specific growth factors, such as TGF- $\beta$ , may cause epithelial cell death (Kyrianiou and Isaacs 1989; Warner et al. 1991; Chung et al. 1992). However, the fact that progesterone antagonists are

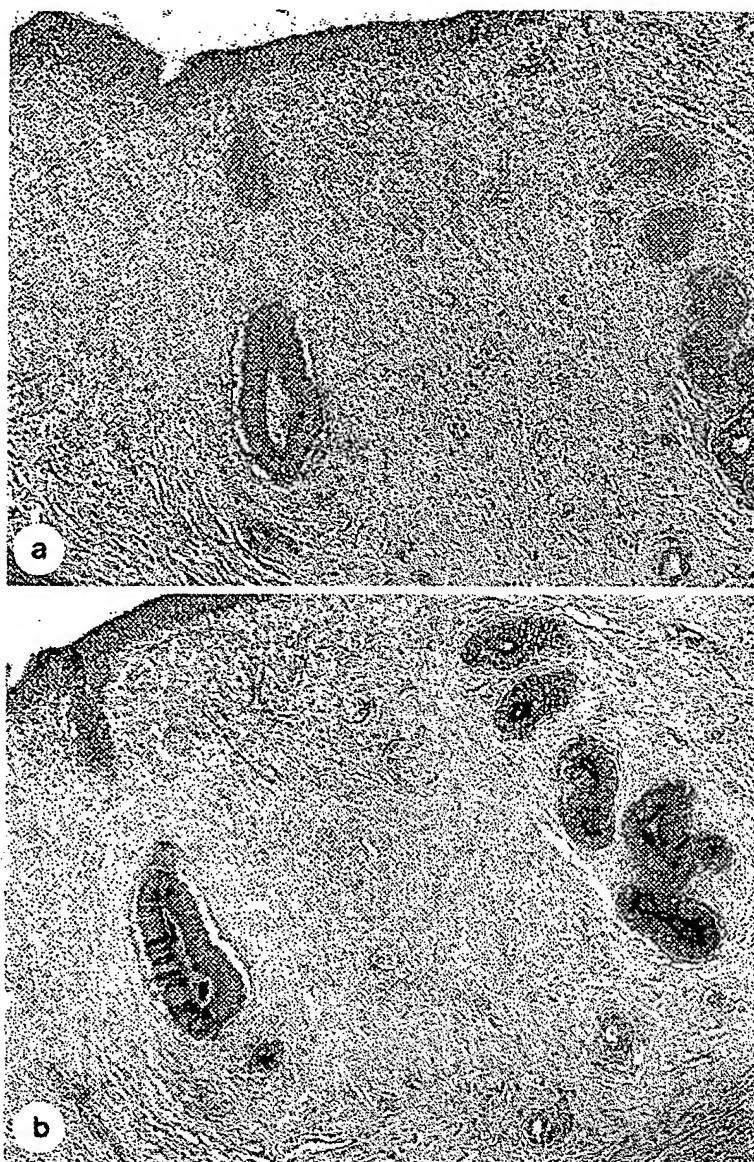


Fig. 9a,b. Legend see p. 175

#### Differentiation and Apoptosis

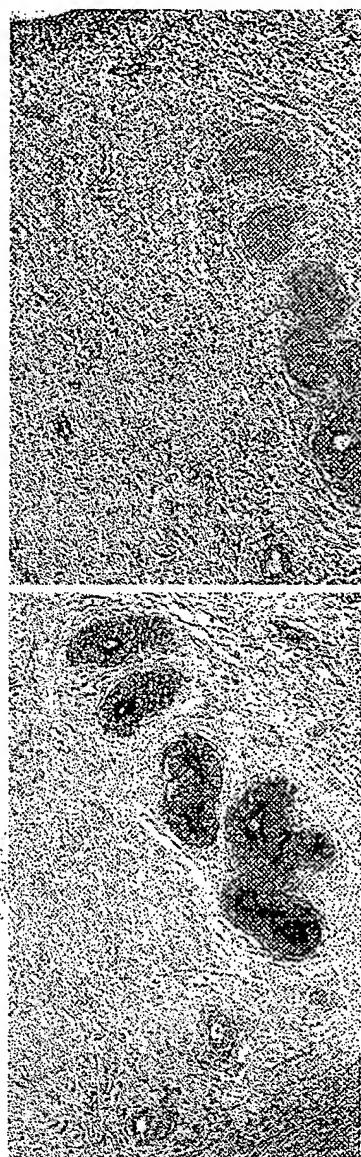
able to exert these effects even the question as to how the b ganded progesterone receptor differentiation and apoptosis v

Most astonishingly, in ac drogen receptor antagonists i regressing prostates, although tiation of the prostate tissue (f other antihormones, tamoxif 1991; Vignon and Rocheft (Chap. 10, this volume), as we al.1994) are capable of inducir the degree of differentiation. I the only compound class whi triggering apoptosis and diffe cinomas in vivo. It would be i mechanisms play a role in th cancer treatment.

In terms of using drugs therapeutic principal one nee effects and the goal to achiev are data indicating that, pos expression of tumor cells (Vat cells seem to be more likely t than normal cells, which has these cells have to pay for t Cotter 1994).

An exciting strategy for the independent cancers, would it act with the cell suicide mecha question then is the choice of death induction: Theoreticall factors *inducing* cell death or cell death (e.g., blockade of b

◀ Fig. 9a,b. Focal expression of T ment with progesterone antagoni



able to exert these effects even in the absence of progesterone still raises the question as to how the binding of the antiprogestins to the unliganded progesterone receptor can produce the pronounced effects of differentiation and apoptosis which result in strong tumor inhibition.

Most astonishingly, in addition to progesterone antagonists, androgen receptor antagonists may also induce apoptotic cell death in regressing prostates, although this process is judged to be a dedifferentiation of the prostate tissue (Aumüller 1979). It thus appears that many other antihormones, tamoxifen-like antiestrogens (Kyprianou et al. 1991; Vignon and Rochefort 1994), and vitamin D<sub>3</sub> analogues (Chap. 10, this volume), as well as chemotherapeutic agents (Walker et al. 1994) are capable of inducing apoptosis independent of their effect on the degree of differentiation. However, the progesterone antagonists are the only compound class which, in our hands, seem to be capable of triggering apoptosis and differentiation in experimental mammary carcinomas *in vivo*. It would be interesting to know whether both of these mechanisms play a role in the clinical use of antiprogestins in breast cancer treatment.

In terms of using drugs which induce apoptotic cell death as a therapeutic principal one needs to consider the issue of possible side effects and the goal to achieve selectivity in killing tumor cells. There are data indicating that, possibly based on high levels of oncogene expression of tumor cells (Vaux et al. 1988; Reed et al. 1990), malignant cells seem to be more likely to be susceptible to undergoing apoptosis than normal cells, which has already been considered to be the price these cells have to pay for their proliferative advantage (Martin and Cotter 1994).

An exciting strategy for the treatment of cancer, especially hormone-independent cancers, would therefore be to create agents that can interact with the cell suicide mechanism of neoplastic cells. The most crucial question then is the choice of a suitable target for tumor-selective cell death induction: Theoretically, either one could consider activating factors *inducing* cell death or the blockade of a factor that *protects* from cell death (e.g., blockade of bcl-2 gene expression or protein function).

Fig. 9a,b. Focal expression of TRPM-2 in uterine epithelial cells after treatment with progesterone antagonists (b); the IgG control is shown in a

**Table 3.** Proteins known to promote and prevent apoptotic cell death and which, therefore, may serve as targets for a therapeutic strategy based on apoptosis

Proteins involved in cell death	Function
ICE (CED-3)	Promotes death
TRPM-2	Promotes/prevents death?
BCL-2 (CED-9)	Prevents death
BAX	Promotes death
p53	Promotes death
FAS/APO1-ligand	Promotes death
BCL-X long	Prevents death
BCL-X short	Promotes death
c-Myc	Promotes death
TNF	Promotes death
TGF- $\beta$	Promotes death
Estrogen/androgen/gestagens	Prevent death

The approach to induce cell death of genuine cell death genes or by a receptor for cell death command; reviewing the targets involved in a growing number of proteins involved appears that their function may also (Bissonette et al. 1994), most probably, less, the induction of active cell death effective strategy for the treatment (Kyprianou et al. 1994).

As for the clinical use of progestins based on the data presented here reported on the beneficial outcome of ovariectomy (Schinzinger 1889), represents a promising future.

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BCL-X short	Opposes/binds BCL-X long
c-Myc	Promotes even proliferation
TNF	Ligand
TGF- $\beta$	Ligand
Estrogen/androgen/gestagens	Stimulate proliferation of hormone dependent tissues

The approach to induce cell death could either be achieved by the design of genuine cell death genes or by identification of a ligand that activates a receptor for cell death command (e.g., FAS/APO-1 ligand). When reviewing the targets involved in cell death there seem to be not only a growing number of proteins involved in cell death (Table 3), but it also appears that their function may also be dependent on interactions (Bissonette et al. 1994), most probably within a cascade system. Nevertheless, the induction of active cell death by apoptosis may provide a new effective strategy for the treatment of even hormone-relapsed tumors (Kyprianou et al. 1994).

As for the clinical use of progesterone antagonists, one can conclude based on the data presented here that, a century after Schinzingher reported on the beneficial outcome of ovarian hormone depletion after ovariectomy (Schinzingher 1889), an innovative hormone treatment represents a promising future.

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10.1	Introduction .....
10.2	Results and Discussion .....
10.2.1	Evidence for Induction .....
10.2.2	Effects of Vitamin D on Estrogen Receptor .....
10.3	Conclusion .....
	References .....

### 10.1 Introduction

Maintenance of normal tissue requires a balance between the rate of cell division and the rate of cell death. The controlled growth characteristic of normal tissues may be due to either an increase in the rate of cell division or a decrease in the rate of cell death, or both. Cell death may be classified into one of two categories: necrosis or apoptosis, or programmed cell death, or cellular self-destruction. Apoptosis is a series of distinct steps involving the death of cells in the prostate, mammary gland, and other tissues (Kroemer and Palek, 1990). A schematic representation of ACD is shown in Fig. 1.

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**From:** Davis, Minh-Tam  
**Sent:** Friday, September 01, 2006 11:51 AM  
**To:** STIC-ILL  
**Subject:** FW: Reprint request for 10/077435

Please add:

1) Michna, H, 1989, J Steroid Biochem Mol Biol, 34: 447-453.  
2) Schneider MR, 1990, J Steroid Biochem Mol Biol, 1990, 37: 783-787

-----Original Message-----

**From:** Davis, Minh-Tam  
**Sent:** Friday, September 01, 2006 10:59 AM  
**To:** STIC-ILL  
**Subject:** Reprint request for 10/077435

- 1) Tenniswood M, eds, Apoptosis in hormone-dependent cancers, Berlin and Heidelberg, Springer Verlag, 1995, pages 161-180.
- 2) El Etreby MF, 1998, Breast cancer res treat, 51: 149-168.
- 3) El Etreby MF, 1998, Breast cancer res Treat, 49: 109-117.
- 4) Parczky, K, 1996, J Cancer Res Clin Oncol, 122: 283-396.

Thank you.

MINH TAM DAVIS  
ART UNIT 1642, ROOM 3A24, MB 3C18  
272-0830

## ANTITUMOR ACTIVITY AND MECHANISM OF ACTION OF DIFFERENT ANTIPOGESTINS IN EXPERIMENTAL BREAST CANCER MODELS

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**Summary**—Onapristone and other antiprogestins proved to possess a potent antitumor activity in several hormone-dependent experimental breast cancer models. This activity is as strong or even better than that of tamoxifen or ovariectomy in the MXT-mammary tumor of the mouse and the DMBA- and MNH-induced mammary tumor of the rat. The antitumor activity is evident in these models in spite of elevated serum levels of ovarian and pituitary hormones. The detailed analysis of all our data including the morphological (ultrastructure) studies of the mammary tumors of treated animals and the effects on growth and cell cycle kinetics using DNA flow cytometry indicates that the antitumor action of antiprogestins is mediated via the progesterone receptor and related to the induction of terminal cell differentiation leading to increased cell death. The strong antitumor activity of antiprogestins in our experimental breast cancer models does not primarily depend on a classical anti-hormonal mechanism. The antiprogestin-related reduction of the number of mammary tumor cells in the S-phase in our experimental tumor models ( $G_0G_1$  arrest) emphasizes the unique innovative mechanism of action of these new agents in the treatment of human breast cancer.

### INTRODUCTION

First choice for endocrine therapy of advanced postmenopausal breast cancer is the antiestrogen tamoxifen, whilst the second choice falls on the use of high-dose progestins and amino-glutethimide. A new approach for treatment of breast cancer could be the use of antiprogestins, compounds which were developed originally for the inhibition of progesterone-dependent processes as, for example, for interruption of pregnancy. First studies in progesterone receptor positive mammary carcinoma cell lines showed, however, that the antiprogestin, Mifepristone (RU 486, Fig. 1), had an inhibitory effect on cell growth [1] and it also proved to have a growth-inhibiting effect on the DMBA-induced mammary carcinoma of the rat [2]. In a preliminary clinical trial with heavily-pretreated patients some did respond to treatment with RU 486 [3].

Whilst the natural configuration of the steroid skeleton is maintained in Mifepristone (Roussel Uclaf), a further group of antiprogestins has been developed in which the link between the C and D rings is *cis* rather than *trans*. Furthest

developed of the compounds in this group is Onapristone (ZK 98.299, Schering AG), which has a somewhat stronger antiprogestagenic effect but reduced antiglucocorticoid activity when compared to Mifepristone [4].

In order to obtain an insight into the tumor-inhibiting potential of this new class of compounds the effect of this antiprogestin was compared with that of Mifepristone in relevant experimental mammary carcinomas. The intention was also to discover the mechanism of action underlying the tumor-inhibiting effect of antiprogestins, particularly because estrogens, but to a lesser degree, progestins are known to be responsible for the growth of hormone-dependent mammary carcinomas. Therefore, Onapristone (ZK 98.299) and Mifepristone (RU 486) were compared with standard mammary carcinomas therapies in a number of different experimental arrangements with the hormone-dependent transplantable MXT(+)-mammary tumor of the mouse as well as with DMBA- and MNH-induced mammary carcinomas of the rat. To clarify the mechanism of action of antiprogestins their effect on endocrine-dependent organs and their histology as well as on the morphologic reaction pattern of these tumors was investigated by means of light and electron microscopy. Moreover, a study on cell cycle kinetics of mammary tumors treated by various

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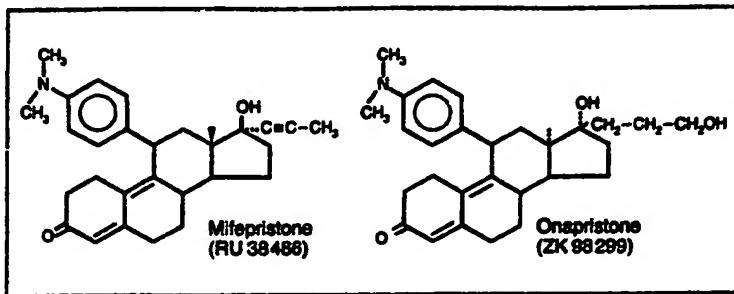


Fig. 1. Chemical structure of Onapristone (Schering AG) and Mifepristone (Roussel-Uclaf).

endocrine therapies including antiprogestins was performed. The importance of hormone deprivation and the importance of a mechanism of action unknown up to now for induction of the antiprogestin tumor-inhibiting potency was investigated in detailed studies with MXT(+) mammary tumors in ovariectomized, hormone-substituted mice.

#### INHIBITORY EFFECT ON EXPERIMENTAL MAMMARY TUMORS

##### *Hormone-dependent MXT(+)mammary tumor of the mouse*

The antiprogestin Onapristone (5 mg/kg) was first compared with tamoxifen (5 mg/kg), and diethylstilbestrol (2.5 mg/kg) as a high-dose estrogen therapy and ovariectomy for its tumor-inhibiting effect on the hormone-dependent, estrogen and progesterone receptor positive MXT(+)mammary tumor in the mouse. In the experimental system employed (prophylaxis model) treatment started immediately after implantation of 2 tumors per mouse and was carried out over a period of 6 weeks. Treatment with tamoxifen and DES resulted only in a retardation of tumor growth as compared with the intact control. Onapristone had a pronounced antitumor effect and caused a strong inhibition of tumor growth comparable to that after ovariectomy (Fig. 2).

Because of this considerable effect an experimental arrangement was chosen in which treatment was started 3 weeks after implantation of the tumors (therapy of established tumors). In this experiment treatment with tamoxifen (4 mg/kg) for 3 weeks again led to a retardation of tumor growth, high-dose progestins (medroxyprogesterone acetate, 100 mg/kg, megestrol acetate, 25 and 50 mg/kg) had no significant effect, whereas ovariectomy resulted in tumor inhibition of 70%. Onapristone and Mifepristone were tested in doses of 1 and 10 mg/kg.

Onapristone and Mifepristone had a strong antitumor effect comparable to that of ovariectomy and significantly better than that of tamoxifen [5]. In a further study on established MXT(+)tumors over a period of only 2 weeks the two antiprogestins were tested against tamoxifen (10 mg/kg) in doses of 50 mg/kg (Fig. 3). The antiestrogen again had only a weak inhibitory effect; but the two antiprogestins achieved an inhibitory effect even superior to that of ovariectomy [5]. This result is all the more remarkable because endogenous estrogens are generally considered responsible for the growth of hormone-dependent mammary carcinomas, progestins only to a secondary extent.

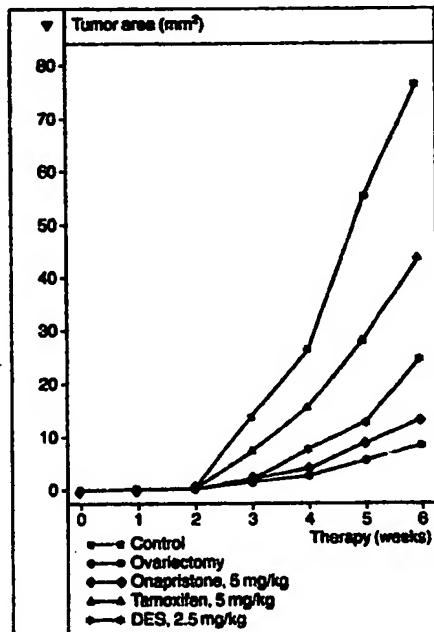


Fig. 2. Effect of Onapristone, tamoxifen, diethylstilbestrol (DES), and of ovariectomy on growth of the hormone dependent MXT(+)mammary tumor of the mouse (prophylaxis model). Compounds were administered six times weekly s.c. for 6 weeks.

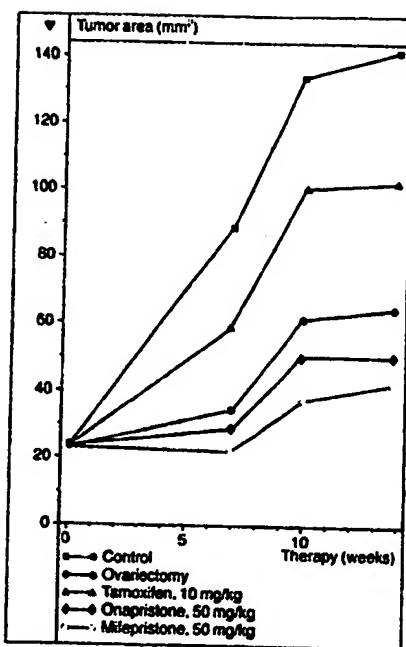


Fig. 3. Effect of Onapristone, Mifepristone and of ovariectomy on growth of established MXT(+)-mammary tumors of the mouse. Compounds were administered six times weekly s.c. for 2 weeks.

#### Hormone-dependent DMBA- and NMU-induced mammary carcinoma in the rat

The dimethylbenzanthracene (DMBA)-induced mammary carcinoma of the rat was used to compare the two antiprogestins in doses of 10 mg/kg daily s.c. with ovariectomy [5]. Four to 8 weeks following induction with 10 mg DMBA, the animals were allocated to individual test groups if their largest tumor has a surface area of at least 150 mm<sup>2</sup>. Treatment of established tumors was performed for 4 weeks. Whilst tumor growth was progressive in the intact controls ovariectomy resulted in almost complete regression of the tumors. Treatment with Onapristone caused strong and very uniform inhibition of tumor growth almost comparable to that after ovariectomy, whereas therapy with Mifepristone resulted in an inhomogeneous tumor inhibition [5].

Both antiprogestins were further compared with ovariectomy using the nitrosomethyl urea (NMU)-induced mammary carcinoma [5]. This tumor differs from the DMBA-mammary carcinoma in its lesser degree of prolactin-dependence and its more aggressive growth. Seven to 14 weeks after induction by a single i.v. injection of 50 mg/kg of NMU the animals were allocated to groups as in the DMBA model. Treatment of established tumors is carried out

over a period of 3 or 6 weeks. In contrast to the progressive growth in the intact controls, ovariectomy led to complete inhibition of the tumor. Therapy with 10 mg/kg Mifepristone led only to a non-significant retardation of tumor growth so that the animals had to be removed from the trial after 3 weeks (Fig. 4). Onapristone (10 mg/kg), however, caused a marked and highly significant tumor inhibition which enabled treatment to proceed for 6 weeks as it did with the ovariectomized animals [5].

To summarize, these antiprogestins have pronounced tumor-inhibiting effects in a number of different hormone-dependent mammary carcinoma models. These effects are superior to those of tamoxifen and high-dose progestins and almost equal to the effect of ovariectomy.

#### MECHANISM OF TUMOR INHIBITION

According to theoretical considerations the mechanism of the tumor inhibiting potency of antiprogestins can depend on the following possibilities:

Antagonism of the effect of progesterone: classical antihormonal (= "antiprogestagenic") action;

Blocking of pituitary and ovary function (antagonadotrophic activity);

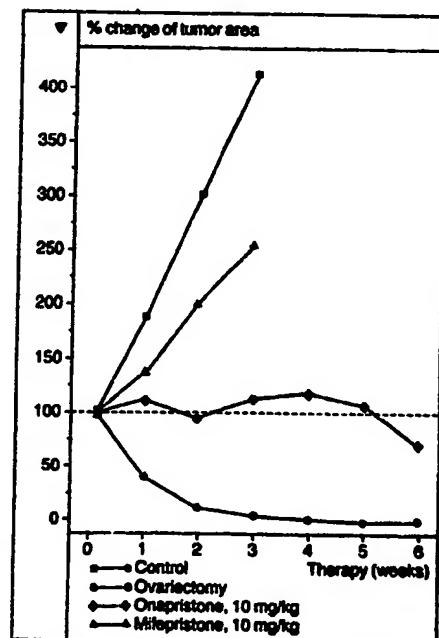


Fig. 4. Effect of Onapristone, Mifepristone and of ovariectomy on growth of hormone-dependent, NMU-induced mammary tumors of the SD-rat. Compounds were administered six times weekly s.c.

**Non-receptor-mediated cytotoxic effects; and  
Progesterone receptor-mediated blockade  
of tumor cell growth.**

In the following studies these possibilities were systematically examined.

After treatment of the mammary carcinoma-bearing animals with Onapristone and Mifepristone an exact analysis of their effect on endocrine parameters was carried out [5, 6]. The studies revealed a stimulation of the pituitary and ovary function recorded in the activation of morphologic parameters (weight, number and histology of the corpora lutea) and increased levels of pituitary (prolactin, LH) and ovarian (estradiol, progesterone) hormones. Therefore, the tumor-inhibiting effect of the antiprogestins is not based on the blocking of pituitary and ovary function [6]. As a reaction to the activation of the ovary functions the target organs, uterus, vagina and mamma, exhibited characteristic estrogen-dependent features. In the light of these estrogenic reactions the simultaneous inhibition of hormone-dependent mammary carcinomas after administration of antiprogestins was even more surprising.

This prompted enquiry into whether the action of antiprogestins is based on non-receptor-mediated cytotoxic effects. Tests in the hormone-independent, receptor negative MXT-OVEX mammary carcinoma of the mouse revealed no indication of tumor inhibition by non-receptor-mediated cytotoxicity [6].

Important clues to the mechanism of action arose from studies with ovariectomized animals following hormonal substitution with an estrogen and/or progestin and simultaneous administration of the antiprogestin Onapristone [7]. The hormone-dependent MXT(+) tumor model of the mouse was chosen for these investigations. Hormone substitution was carried out on the day following tumor implantation. On substitution with a progestin (medroxyprogesterone acetate) in ovariectomized animals tumor growth was only marginally stimulated above the low level found with ovariectomy alone. This provides a first indication that hormone deprivation hardly contributes to any great extent to the tumor inhibiting capacity of antiprogestins. In fact the slight stimulation by the progestin can only be influenced minimally by simultaneous administration of the antiprogestin Onapristone [7]. In substitution experiments with estrogen (estradiol benzoate) — in

contrast to substitution with progestins — tumor growth in ovariectomized animals was stimulated up to the level of intact controls [7] (Fig. 5). Surprisingly, this effect was completely antagonized by antiprogestins, even though no progestins were present in this experimental arrangement. This result shows that the tumor inhibiting mechanism of the antiprogestins cannot primarily depend on a classical anti-hormonal (=antiprogestagenic) effect or to progesterone deprivation. As the progesterone receptor content in mammary carcinomas is known to be strongly induced by administration of estrogens, the tumor inhibiting mechanism of antiprogestins depends on a progesterone receptor-mediated, as yet unknown effect. Further experiments with substitution of an estrogen and a progestagen in ovariectomized mice with MXT(+) mammary carcinomas underline the importance of the progesterone receptor for the induction of tumor inhibition by antiprogestins [7]. These experiments with different concentrations of a progestin clearly reveal that a tumor inhibiting activity of antiprogestins is only given if a sufficient concentration of available progesterone receptors is present. This was also demonstrable using the human postmenopausal T61 mammary tumor implanted in nude mice. Only after stimulation of the apparently low progesterone receptor content by a

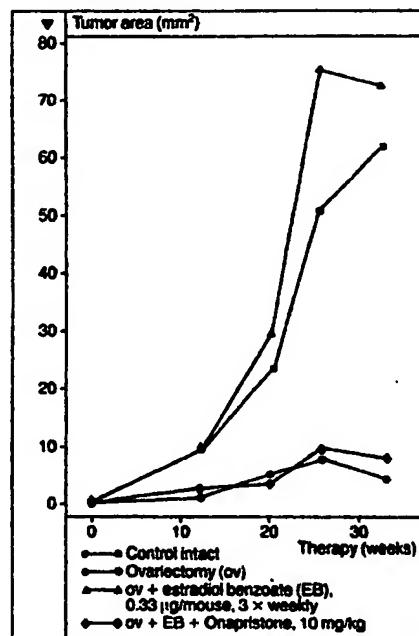


Fig. 5. Effect of Onapristone (six times weekly s.c.) on growth of MXT(+) mammary tumors in ovariectomized, estradiol benzoate-substituted mice.

low dose of estrogen, a tumor-inhibiting effect of antiprogestins was given [8].

In these estrogen (and progestin) substitution experiments as well as in all experiments in intact animals (MXT-, DMBA-, NMU-tumors) detailed light and electron microscopic studies of the tumors revealed that after administration of antiprogestins the morphology was clearly distinguishable from that after ovariectomy [5-7]. Whilst necrobiotic degenerations are the characteristic features of mammary carcinomas following ovariectomy, those mammary carcinomas whose growth has been inhibited by antiprogestins display clear signs of differentiation. The outstanding feature of their morphology is the massive presence of acinar nodules which regularly are secretory activated. As regards the morphology of the mammary carcinomas in intact and ovariectomized animals, all the light and electron microscopy findings in these tumor experiments support a concept of the induction of terminal differentiation in progesterone receptor positive mammary carcinomas after administration of antiprogestins. These findings show that a differentiation of polygonal, actively dividing individual cells to highly secretory, mitotically inactive dysplastic acini and glandular ducts takes place. It is well-known that in a large number of tissues the relationship between proliferation and differentiation—here: the performance of specific cell functions—is one of mutual exclusion. After loss of cell contact the epithelial cells of these acini regularly undergo cell death. The appearance of apoptoses may also be considered as a typical feature of programmed cell death. Thus, these tumor cells go through the complete physiological differentiation program after proliferation commences.

The treatment of experimental hormone dependent mammary tumors with antiprogestins induced an accumulation of cells in  $G_0$ ,  $G_1$  phase together with a significant and biologically relevant reduction in the number of cells in the S and  $G_2$ , M phase [9]. Interestingly, there are observations in some stem cell types that hormonal control of  $G_1$  and cell differentiation are somehow linked and a differentiation specific arrest was already proposed. Keeping this in mind, the accumulation of the tumor cells in  $G_0$ ,  $G_1$  may display differentiation and this correlates with all our quantitative light- and electron-microscopical data indicating that the antitumor action of antiprogestin is

mainly related to the induction of terminal differentiation leading to cell death.

We conclude that the tumor inhibiting capacity of antiprogestins is based on their ability to trigger the mechanism of terminal differentiation probably by removing the intrinsic block at the genomic site. In light of the tumor experiments conducted, this action of the antiprogestins depends on the presence of a sufficient number of available progesterone receptors.

In consequence, in the clinical situation only progesterone receptor positive breast cancer patients should be selected for therapy with antiprogestins. According to the data obtained for the tumor inhibiting potency and mechanism of action of antiprogestins, these compounds are expected to provide an innovative and effective therapy for hormone dependent breast cancer.

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6. Michna H., Schneider M. R., Nishino Y. and El Etreby M. F.: Antitumor activity of the antiprogestins ZK 98.299 and RU 38.486 in hormone dependent rat and mouse mammary tumors: mechanistic studies. *Breast Cancer Res. Treat.* 14 (1989) 275-288.
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9. Michna H., Schneider M. R., Nishino Y., El Etreby M. F. and McGuire W. L.: Progesterone antagonists block the growth of experimental mammary tumors in the cell cycle. *Breast Cancer Res. Treat.* In press.



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**STIC-ILL**

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**From:** Davis, Minh-Tam  
**Sent:** Friday, September 01, 2006 10:59 AM  
**To:** STIC-ILL  
**Subject:** Reprint request for 10/077435

1) Tenniswood M, eds, Apoptosis in hormone-dependent cancers, Berlin and Heidelberg, Springer Verlag, 1995, pages 161-180.

2) El Etreby MF, 1998, Breast cancer res treat, 51: 149-168

3) El Etreby MF, 1998, Breast cancer res Treat, 49: 109-117.

4) Parczky, K, 1996, J Cancer Res Clin Oncol, 122: 283-396.

Thank you.

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272-0830

Report

## Effect of antiprogestins and tamoxifen on growth inhibition of MCF-7 human breast cancer cells in nude mice

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**Key words:** antiestrogens (tamoxifen), antiprogestins (mifepristone, onapristone), breast cancer, MCF-7 cells, nude mice

### Summary

This is the first report demonstrating an *in vivo* antitumor activity of antiprogestins (mifepristone, onapristone) alone and in combination with tamoxifen in the MCF-7 human breast cancer model. The MCF-7 cells produced progressive growing tumors in female nude mice supplemented with 17 $\beta$ -estradiol. Tumor regression was observed following either estrogen ablation alone or estrogen ablation in combination with tamoxifen. Monotherapy with tamoxifen or antiprogestins caused a retardation of estrogen-induced tumor progression. Complete inhibition or prevention of tumor growth occurred as a result of simultaneous administration of mifepristone and tamoxifen. The addition of mifepristone in this combination treatment was also effective in delaying or preventing tumor escape (relapse) from the antiestrogenic (antitumor) effect of tamoxifen. These results suggest a potential clinical benefit of adding an antiprogestin to antiestrogen therapy of breast cancer patients.

### Introduction

A new approach for treatment of hormone-dependent breast cancer could be the use of antiprogestins, a new class of compounds (progesterone antagonists) which were developed originally for inhibition of progesterone-dependent processes (e.g. termination of pregnancy) [1]. In preliminary clinical studies, some breast cancer patients responded to treatment with the antiprogestin mifepristone (RU 486, Roussel Uclaf/Population Council). The beneficial effects were observed mainly in patients with progesterone (PR) and estrogen (ER) receptor positive (+) tumors [2–4]. Robertson et al. [5] have conducted a clinical study using the antiprogestin onapristone (ZK 98.299, Schering

AG-100 mg/day) as a first-line endocrine therapy in either patients with locally advanced breast cancer (n = 12) or elderly patients with primary metastatic disease (n = 7). Seventeen of the 19 tumors expressed estrogen receptors. 56% of treated patients showed partial remission and 11% showed a static disease, giving an overall tumor remission rate of 67% [5].

While the antitumor activity of antiprogestins in rodent mammary tumor models has been the subject of intense research [2, 6–14], their effect in *in vivo* human breast cancer models has received much less attention [9, 12]. The authors could not find any published data demonstrating that antiprogestins will inhibit the hormone-dependent ER (+) and PR (+) MCF-7 human breast cancer model

in nude mice. This human breast cancer model system has been used frequently to test the antitumor activity and mechanism of action of estrogen ablation and antiestrogen treatment [15–20]. Therefore, our objective in this study was to compare the antitumor activity of antiprogestins (mifepristone, onapristone) with that of tamoxifen or the combination of both utilizing the MCF-7 human breast cancer cell line inoculated into female nude mice supplemented with  $17\beta$  estradiol. The effect of estrogen withdrawal (ablation) alone and in combination with tamoxifen treatment was also determined to evaluate the contribution of the antihormone (antiestrogen/antiprogestin) effect versus estrogen ablation. In this paper, results are presented to demonstrate for the first time an antitumor activity of antiprogestins alone and in combination with tamoxifen in the MCF-7 human breast cancer model in nude mice.

## Materials and methods

### *Cell line and tumor growth*

The MCF-7 human breast cancer cells (passage number 149) were obtained from American Type Culture Collection (ATCC), Rockville, MD. The cells were cultured in Dulbecco's Modified Eagle medium (DMEM), low glucose, containing 1 mM sodium pyruvate, 10  $\mu$ g/ml bovine insulin, and 10% fetal bovine serum. They were maintained at 37° C, 5% CO<sub>2</sub> incubator. The MCF-7 cells ( $5 \times 10^6$  cells) were inoculated subcutaneously (s.c.) into the right flank of 6- to 8-week old intact female nude mice (BALB/c, nu/nu; Harlan, Indianapolis, IN). One day before inoculation of MCF-7 cells, the animals were implanted with  $17\beta$ -estradiol (E<sub>2</sub>) pellets (Innovative Research of America, Sarasota, FL) into the left flank. Different E<sub>2</sub> pellets were used in the various experiments (0.72 or 1.7 mg E<sub>2</sub>/pellet for 60 or 90 days release) to maintain a constant high serum E<sub>2</sub> level for the duration of the experiments (see Results). The nude mice were examined for tumors by palpation. Tumor size was measured in three dimensions in millimeters once or twice weekly and tumor volumes were calculated accord-

ing to the known formula: Volume =  $\pi/6 \times [\text{Length} \times \text{Width} \times \text{Height}]$  and expressed in cubic millimeters ( $\pi = 3.1416$ ). In all experiments, caliper measurement of tumor volume was performed in a blinded fashion (without knowledge of the different experimental groups) by two independent investigators. The tumors were permitted to grow in the presence of the E<sub>2</sub> pellets until they reached a volume of about 160–350 mm<sup>3</sup>. The initial tumor volume (at the start of treatment) was different in our various experiments. This variable, as well as other specific conditions (e.g. E<sub>2</sub> pellets, number of tumor-bearing animals, etc.) of each experiment, are given in the legends of the figures, in the tables, and/or in the results of the different nude mouse experiments.

### *Experimental design*

The animals were randomly assigned to different experimental groups of 3–5 animals each. A positive control group retained the E<sub>2</sub> pellets to allow continued tumor growth. E<sub>2</sub> pellets were removed in some groups to inhibit tumor growth (estrogen ablation) by puncturing the skin and retrieving the pellet with forceps. The other groups retained the E<sub>2</sub> pellets and were treated with mifepristone, onapristone (both generously provided by Schering AG, Berlin, Germany), tamoxifen, or their combinations. The treatments were continued for either 17 or 35 days (5 weeks) in various experiments. The animals in the antiprogestin treatment groups received mifepristone (50 mg/kg/day s.c.) or onapristone (30 mg/kg/day s.c.) suspended in 0.1 ml vehicle (0.1 ml ethanol, 0.0625 ml Tween 80, and 0.8375 ml 0.9% NaCl). The animals in the tamoxifen treatment groups received a tamoxifen pellet (15 mg/pellet) for 60 days release of 10 mg/kg/day (Innovative Research of America, Sarasota, FL). Control animals and animals in the tamoxifen monotherapy groups were treated daily with 0.1 ml of the above-mentioned vehicle s.c. To test the estrogen dependency of our MCF-7 tumor model system, a few animals were implanted with the E<sub>2</sub> pellets to allow continuous tumor progression, then the E<sub>2</sub> pellets were removed. Tumor regression was followed by

caliper measurement of tumor volume once weekly as described above. After the tumors became unpalpable for different periods of time (up to several months), reimplantation of the  $E_2$  pellets was performed on these same animals to study the effect of estrogen-resubstitution on tumor growth. These cycles of  $E_2$  pellet supplementation and  $E_2$  pellet removal were repeated several times. At the end of the experiments, all animals were sacrificed by spinal elongation and the tumors were harvested and wet weights determined. Percentage inhibition of tumor volume or tumor weight was calculated according to the formula:  $T-C/C \times 100$  ( $T$  = treatment,  $C$  = Control).

#### *Statistical analysis*

Differences among groups for the antitumor activity were tested using one-way analysis of variance (ANOVA) with repeated measures over time. The assumption of analysis of variance was examined and nonparametric tests based on ranks used if needed. Values for tumor volume and tumor weight were reported as means  $\pm$  standard errors of the mean (SEM). When ANOVA indicated significant treatment effects ( $F$ -ratio,  $P < 0.05$ ), the Student-Newman-Keuls multirange test was employed to compare individual treatment means.

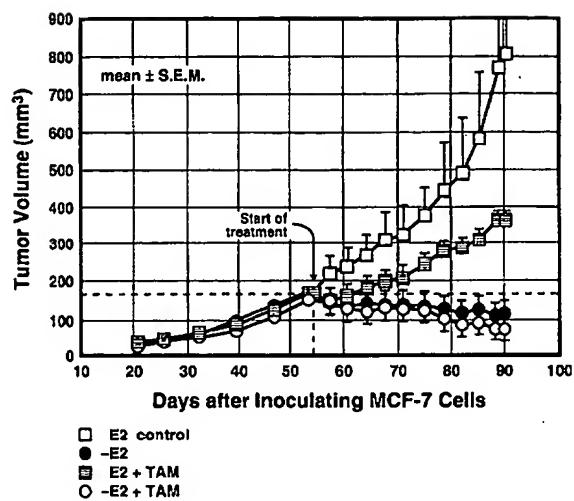
## **Results**

#### *Effect of estrogen supplementation, estrogen ablation, and/or tamoxifen treatment*

In a first experiment to test the estrogen-dependency of our MCF-7 tumor model system, the MCF-7 tumors showed a slow growth pattern in the presence of  $E_2$  pellets (0.72 mg  $E_2$ /pellet to maintain serum  $E_2$  levels between 300–400 pg/ml for 60 days). These tumors were permitted to grow for almost 8 weeks after subcutaneous injection of the MCF-7 cells in nude mice until they reached an average tumor volume of about 160 mm<sup>3</sup>. In the control group,  $E_2$  supplementation induced a significant time-dependent tumor progression. The average tumor

volume increased from about 160 mm<sup>3</sup> (at start of treatment) to about 800 mm<sup>3</sup> after 5 weeks of continuous estrogen stimulation. Tamoxifen (10 mg/kg/day) caused a retardation of the  $E_2$ -induced tumor progression. At the end of treatment, tamoxifen induced approximately 50% inhibition of tumor growth. However, this inhibition was not significantly different ( $P > 0.05$ ) as compared with the  $E_2$  control group (Figure 1 and Table 1). In the group of animals in which the  $E_2$  pellets were removed, estrogen ablation (withdrawal) induced 86 or 88% inhibition of tumor growth as compared with the  $E_2$  control group ( $P < 0.05$ ). The combination of tamoxifen treatment and estrogen withdrawal resulted in slightly more tumor growth inhibition as compared with estrogen ablation alone. However, the extent of inhibition of tumor volume and tumor weight in this combination group was only significantly different ( $P < 0.05$ ) as compared with the  $E_2$  control group (see Table 1). In all treatment groups (except the  $E_2$  control), a slight decrease in tumor volume (tumor regression) was observed for the first few days of treatment. This tumor regression continued in both groups treated by estrogen ablation alone or estrogen ablation in combination with tamoxifen. The average tumor volume in these two groups at the end of the 5 weeks of treatment was 113 or 78 mm<sup>3</sup>, clearly below the initial tumor volume at the start of treatment (about 160 mm<sup>3</sup>). On the contrary, in the group treated with tamoxifen alone, the transient tumor regression was followed with a progressive increase in tumor volume (relapse), indicating an escape from the (antiestrogen) antitumor effect of tamoxifen.

The estrogen-dependency of this model was confirmed also by implanting the  $E_2$  pellets in a few animals to allow continued tumor growth for several weeks. When the  $E_2$  pellets were removed tumor growth was inhibited significantly and after about 10 weeks the tumors became unpalpable. After different periods of time (up to several months), the reimplantation of the  $E_2$  pellets caused these tumors to regrow again and become palpable (data not shown). We repeated these  $E_2$  supplementation and  $E_2$  withdrawal cycles several times and the results were reproducible. The tumors disappeared with estrogen withdrawal and growth resumed with



**Figure 1.** Effect of 35 days of estrogen ablation ( $-E_2$ ) and/or tamoxifen (TAM) treatment on the growth of MCF-7 tumors in nude mice. This experiment consisted of the following four groups: (1) a positive control group retaining the  $E_2$  pellets ( $E_2$  control), (2) an estrogen ablation group in which the  $E_2$  pellets were removed ( $-E_2$ ), (3) a tamoxifen treatment group retaining the  $E_2$  pellets and receiving a tamoxifen pellet (15 mg/pellet) for 60 days release of 10 mg/kg/day ( $E_2 + TAM$ ), and (4) an  $E_2$ -ablation ( $E_2$  pellets were removed) group which also received the same tamoxifen pellet as described above ( $-E_2 + TAM$ ). Seventeen tumor-bearing mice were included in this experiment.

estrogen supplementation. These results confirm the estrogen-dependency of our MCF-7 tumor model system and clearly indicate that tumor cells remained viable despite prolonged estrogen deprivation. Similar observations were made before [15].

#### *Effect of mifepristone and/or tamoxifen treatment*

In a second experiment, we compared the antitumor activity of mifepristone with that of tamoxifen and the combination of both. The MCF-7 tumors showed a rapid growth pattern in this experiment. These tumors were permitted to grow for only about 3 weeks after subcutaneous inoculation of tumor cells in nude mice (in the presence of the  $E_2$  pellets: 1.7 mg  $E_2$ /pellet to maintain serum  $E_2$  levels above 900 pg/ml for 60 days) until they reached an average tumor volume of about 200 mm<sup>3</sup>. In the control group,  $E_2$  supplementation induced a significant time-dependent rapid tumor progression. The average tumor volume increased from about 200 mm<sup>3</sup> (at start of treatment) up to about 750 mm<sup>3</sup> after 5 weeks of continuous estrogen stimulation. In all treatment groups (except the  $E_2$  control), a slight decrease in initial tumor volume (tumor regression) was observed for the first few days of treatment. In the tamoxifen and mifepristone monotherapy groups, this initial tumor response was followed by a progressive increase in tumor volume (relapse) indicating an escape from the antitumor effect of tamoxifen or mifepristone. Nevertheless, the administration of tamoxifen (10 mg/kg/day) or mifepristone (50 mg/kg/day) as monotherapy, induced a significant retardation of tumor growth. At the end of treatment, monotherapy induced about 50% inhibition of tumor volume as compared with the  $E_2$  control group ( $P < 0.05$ ) (Figure 2 and Table 2). The inhibition of tumor volume

**Table 1.** Effect of estrogen supplementation (17 $\beta$ -estradiol =  $E_2$  control), estrogen ablation ( $-E_2$ ), and/or tamoxifen (TAM) treatment on growth of MCF-7 tumors in nude mice

Groups	Results after 5 weeks of treatment			
	Average tumor volume (mm <sup>3</sup> ) <sup>a</sup>	% Inhibition	Average tumor weight (mg)	% Inhibition
$E_2$ control	801 $\pm$ 311		914 $\pm$ 308	
$E_2 + TAM$	363 $\pm$ 33	55	451 $\pm$ 69	51
$-E_2$ ( $E_2$ ablation)	113 $\pm$ 42 <sup>b</sup>	86	113 $\pm$ 47 <sup>b</sup>	88
$-E_2 + TAM$	78 $\pm$ 16 <sup>b</sup>	90 (31/79) <sup>c</sup>	82 $\pm$ 24 <sup>b</sup>	91 (27/82) <sup>c</sup>

For details of experimental design see Figure 1. Mean  $\pm$  S.E.M.

<sup>a</sup> = Initial average tumor volume at the start of treatment was about 160 mm<sup>3</sup>.

<sup>b</sup> = Significant difference ( $P < 0.05$ ) as compared with  $E_2$  control.

<sup>c</sup> = Numbers between brackets are % inhibition as compared with  $E_2$  ablation or  $E_2 + TAM$  groups.

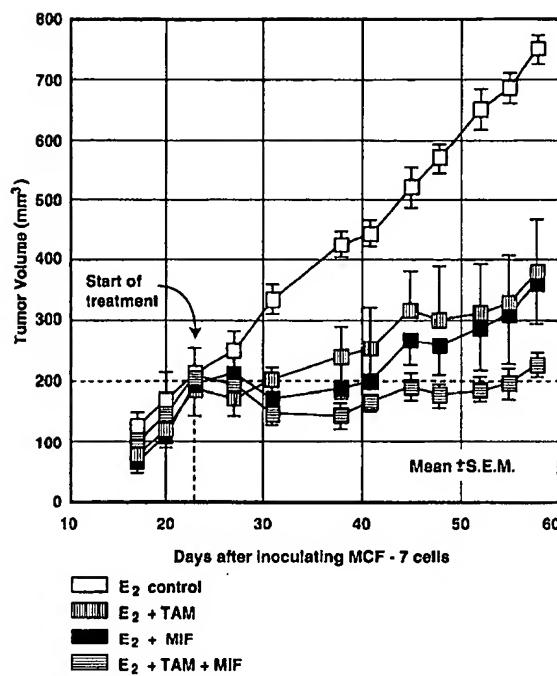


Figure 2. Effect of 35 days of treatment with tamoxifen (TAM), mifepristone (MIF), and their combination on estrogen ( $17\beta$ -estradiol =  $E_2$ )-induced tumor growth of MCF-7 tumors in nude mice. All animals retained the  $E_2$  pellet and were randomly assigned to 4 groups: (1) a control group receiving the vehicle s.c. ( $E_2$  control), (2) a tamoxifen treated group receiving 15 mg tamoxifen/pellet which is releasing 10 mg/kg/day + the vehicle s.c. ( $E_2$  + TAM), (3) a mifepristone treated group receiving 50 mg/kg/day s.c. ( $E_2$  + MIF), and (4) a combination group treated with the same tamoxifen pellet in combination with mifepristone in the same dose as in group 3 ( $E_2$  + TAM + MIF). Twenty tumor bearing mice were included in this experiment.

and tumor weight was amplified to 70 or 74% by simultaneous administration of tamoxifen and mifepristone. The effect of the various drug treatments on the reduction of tumor volume started to become significantly different ( $p < 0.05$ ) from the control group as early as 8 days after the start of treatment. The antitumor activity in the combination group resulted in complete growth inhibition delaying or preventing the escape phenomenon (relapse) for about 5 weeks. These results suggest that mifepristone (50 mg/kg/day) in combination with tamoxifen (10 mg/kg/day) was slightly more effective as compared with tamoxifen (10 mg/kg/day) or mifepristone (50 mg/kg/day) monotherapy in this particular experiment. However, the extent of inhibition in this combination group was only significantly different ( $P < 0.05$ ) from the  $E_2$  control group (see Table 2).

#### Effect of mifepristone, onapristone, and/or tamoxifen treatment

In a third experiment, the antitumor activity of anti-progestins (mifepristone and onapristone), tamoxifen and their combination was tested. The MCF-7 tumors showed a rapid growth pattern in this experiment in the presence of  $E_2$  pellets (1.7 mg  $E_2$ /pellet to maintain serum  $E_2$  levels at about 500–600 pg/ml for 90 days). These tumors were permitted to grow for about 8 weeks after subcutaneous

Table 2. Effect of tamoxifen (TAM), mifepristone (MIF), and their combination on estrogen-( $17\beta$ -estradiol =  $E_2$ ) induced growth of MCF-7 tumors in nude mice

Groups	Results after 5 weeks of treatment			
	Average tumor volume ( $\text{mm}^3$ ) <sup>a</sup>	% Inhibition	Average tumor weight (mg)	% Inhibition
$E_2$ control	752 ± 17		1013 ± 302	
$E_2$ + TAM	384 ± 115 <sup>b</sup>	49	506 ± 216	51
$E_2$ + MIF	376 ± 188 <sup>b</sup>	50	607 ± 34	40
$E_2$ + TAM + MIF	223 ± 12 <sup>b,c</sup>	70 (42/41) <sup>d</sup>	266 ± 5 <sup>b,c</sup>	74 (47/56) <sup>d</sup>

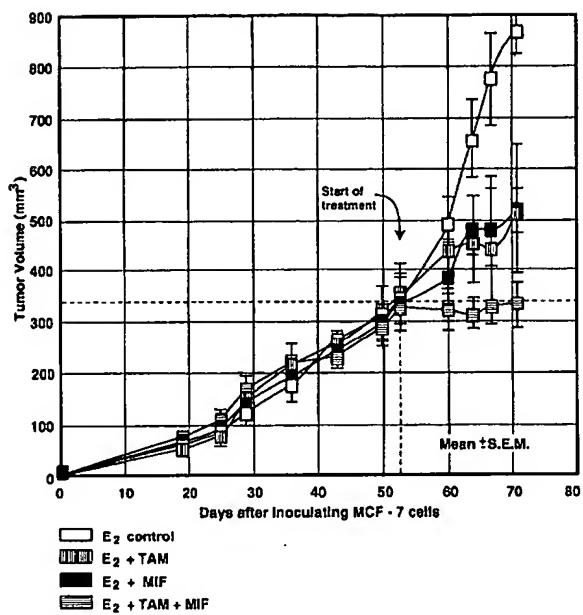
For details of experimental design see Figure 2. Mean ± S.E.M.

<sup>a</sup> = Initial average tumor volume at the start of treatment was about 200  $\text{mm}^3$ .

<sup>b</sup> = Significant difference ( $P < 0.05$ ) as compared with the control group.

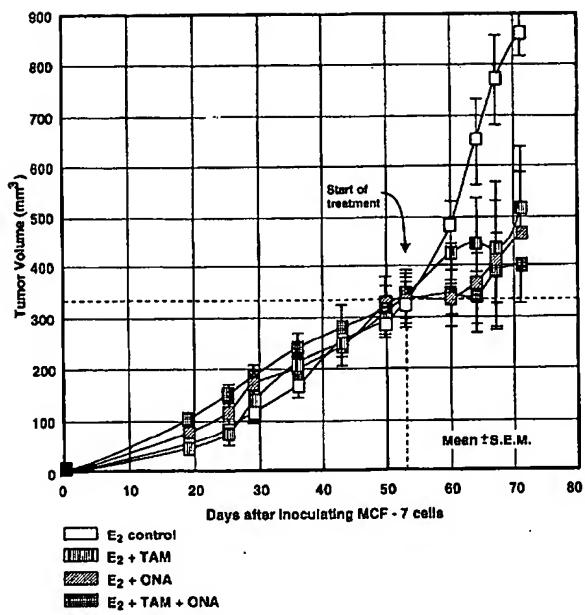
<sup>c</sup> = Difference between combination and single drug treatments is not statistically significant.

<sup>d</sup> = Numbers between brackets are % inhibition as compared with tamoxifen or mifepristone monotherapy group.



**Figure 3.** Effect of 17 days of treatment with tamoxifen (TAM), mifepristone (MIF), and their combination on estrogen ( $17\beta$ -estradiol =  $E_2$ )-induced tumor growth of MCF-7 tumors in nude mice. All animals retained the  $E_2$  pellet and were randomly assigned to 4 groups: (1) a control group receiving the vehicle s.c. ( $E_2$  control), (2) a tamoxifen treated group receiving 15 mg tamoxifen/pellet which is releasing 10 mg/kg/day, + the vehicle s.c. ( $E_2$  + TAM), (3) a mifepristone treated group receiving 50 mg/kg/day s.c. ( $E_2$  + MIF), and (4) a combination group treated with the same tamoxifen pellet in combination with mifepristone in the same dose as in group 3 ( $E_2$  + TAM + MIF). The rest of the results of this experiment are shown in Figure 4. Twenty-six tumor-bearing mice were included in this experiment.

injection of the tumor cells in nude mice until they reached an average tumor volume of about  $350 \text{ mm}^3$ . In the control group,  $E_2$  supplementation induced a significant time-dependent rapid tumor progression. The average tumor volume increased from about  $350 \text{ mm}^3$  (at start of treatment) to about  $860 \text{ mm}^3$  after only 17 days of continuous estrogen stimulation. The administration of tamoxifen (10 mg/kg/day), mifepristone (50 mg/kg/day), or onapristone (30 mg/kg/day) for 17 days as a monotherapy induced approximately 30 or 40% inhibition of tumor growth ( $P < 0.05$ ) as compared with the  $E_2$  control group (Figures 3 and 4 and Table 3). This tumor inhibition was slightly amplified by the simultaneous administration of mifepristone in combination with tamoxifen. This combination treatment resulted in complete growth inhibition of



**Figure 4.** Effect of 17 days treatment with tamoxifen (TAM), onapristone (ONA), and their combination on estrogen ( $17\beta$ -estradiol =  $E_2$ )-induced tumor growth of MCF-7 tumors in nude mice. All animals retained the  $E_2$  pellet and were randomly assigned to 4 groups: (1) a control group receiving the vehicle s.c. ( $E_2$  control), (2) a tamoxifen treated group receiving 15 mg tamoxifen/pellet which is releasing 10 mg/kg/day + the vehicle s.c. ( $E_2$  + TAM), (3) an onapristone treated group receiving 30 mg/kg/day s.c. ( $E_2$  + ONA), and (4) a combination group treated with the same tamoxifen pellet in combination with onapristone in the same dose as in group 3 ( $E_2$  + TAM + ONA). The rest of the results of this experiment are shown in Figure 3. Twenty-six tumor-bearing mice were included in this experiment.

these rapid growing MCF-7 tumors in nude mice (see Figure 3 and Table 3). In this experiment, both tumor volume and tumor weight at the end of the treatment was significantly different ( $P < 0.05$ ) in all treated groups as compared with the control group (see Table 3). Most probably due to the large tumor volume at the start of treatment ( $300$ – $400 \text{ mm}^3$ ) and due to their rapid growth under estrogen stimulation, no treatment-related transient tumor regression was observed. However, the first measurement of tumor volume in this experiment was one week after the start of treatment, so that we may have missed the transient tumor regression phase, which could have been observed within the first few days of treatment.

## Discussion

This is the first report demonstrating an antitumor activity of antiprogestins (mifepristone/onapristone) alone and in combination with tamoxifen in the MCF-7 human breast cancer model in nude mice. Our results were consistent and reproducible in two different experiments, independent of the initial tumor load at the start of treatment and the rate of tumor growth and progression. The estrogen-dependency of our MCF-7 tumor model was clearly evident in all our experiments. In agreement with many previously published studies [15-20], progressively growing tumors were produced only when the animals were supplemented with exogenous E<sub>2</sub>. Without estrogen supplementation, no tumor growth was observed in our intact female nude mice, most probably due to low estrogen levels, unable to support the growth of the MCF-7 tumors. Also, the extent of tumor growth in control animals was different in various experiments, depending on the type of the E<sub>2</sub> pellets used and the serum E<sub>2</sub> levels maintained (see Figures 1-4). Furthermore, in this and other investigations [16-18], tumor regression was observed as a result of estrogen ablation. In our study, the tumor regression as a result of the removal of the E<sub>2</sub> pellets could also be slightly amplified by the simultaneous administration of tamoxifen. On the contrary, in a previous

study estrogen ablation and/or antiestrogen treatment resulted only in cessation of tumor growth, but not in tumor regression [15]. However, other groups reported, in agreement with our results, a rapid reduction in the MCF-7 tumor volume in nude mice treated by estrogen withdrawal and/or tamoxifen [19, 20].

Based on our own previously published studies and in agreement with data of other investigators, the addition of tamoxifen or a pure antiestrogen (ICI 164,384) to the antiprogestin (mifepristone, onapristone, etc.) treatment showed a striking additive antitumor effect in several rodent hormone-dependent breast cancer models [2, 10, 11, 13]. Using the MCF-7 human breast cancer model in nude mice, our data in the present investigations also shows that the combination of tamoxifen and mifepristone completely prevents or abolishes tumor growth, while monotherapy results only in inhibition of tumor growth as compared with the estrogen-treated controls. Moreover, there is a good evidence that the simultaneous administration of mifepristone in combination with tamoxifen is effective in delaying or preventing tumor escape (relapse) from the antiestrogenic (antitumor) effect of tamoxifen. These results mimic the clinical situation and suggest that: (a) adjuvant treatment by antiprogestins in combination with tamoxifen may prevent tumor escape, which usually develops after

Table 3. Effect of tamoxifen (TAM), mifepristone (MIF), onapristone (ONA), and their combinations on estrogen-(17 $\beta$ -estradiol = E<sub>2</sub>) induced growth of MCF-7 tumors in nude mice

Groups	Results after 17 days of treatment			
	Average tumor volume (mm <sup>3</sup> ) <sup>a</sup>	% Inhibition	Average tumor weight (mg)	% Inhibition
E <sub>2</sub> Control	863 $\pm$ 41		1013 $\pm$ 137	
E <sub>2</sub> + TAM	515 $\pm$ 128 <sup>b</sup>	40	605 $\pm$ 185 <sup>b</sup>	40
E <sub>2</sub> + MIF	512 $\pm$ 32 <sup>b</sup>	41	700 $\pm$ 20 <sup>b</sup>	31
E <sub>2</sub> + ONA	464 $\pm$ 117 <sup>b</sup>	46	644 $\pm$ 103 <sup>b</sup>	36
E <sub>2</sub> + TAM + MIF	325 $\pm$ 21 <sup>b,c</sup>	62 (37/37) <sup>d</sup>	465 $\pm$ 22 <sup>b,c</sup>	53 (22/32) <sup>d</sup>
E <sub>2</sub> + TAM + ONA	403 $\pm$ 76 <sup>b,c</sup>	53 (21/13) <sup>d</sup>	576 $\pm$ 34 <sup>b,c</sup>	43 (5/11) <sup>d</sup>

For details of experimental design see Figures 3 and 4. Mean  $\pm$  S.E.M.

<sup>a</sup> = Initial average tumor volume at the start of treatment was about 350 mm<sup>3</sup>.

<sup>b</sup> = Significant difference ( $P < 0.05$ ) as compared with the control group.

<sup>c</sup> = Difference between combination and single drug treatments is not statistically significant.

<sup>d</sup> = Numbers between brackets are % inhibition as compared with tamoxifen or the corresponding antiprogestin monotherapy groups.

a long-term treatment with tamoxifen, and (b) the antitumor effect of tamoxifen can be enhanced by simultaneous administration of antiprogestins. This may increase the rate and magnitude of the clinical response, reduce malignant progression and relapse, as well as increase the survival of breast cancer patients. These assumptions have been recently strengthened by results of *in vitro* studies from our laboratories. In these studies, MCF-7 cells growing in culture were used to explore the mechanism of the antiproliferative activity of mifepristone, as compared with 4-hydroxytamoxifen or the combination of both. These steroid antagonists induced a significant time- and dose-dependent cell growth inhibition (cytotoxicity). This inhibition of cell survival was associated with a significant increase in DNA fragmentation (apoptosis), downregulation of bcl<sub>2</sub>, and induction of TGF $\beta_1$  protein [21]. Abrogation of the mifepristone- and/or 4-hydroxytamoxifen-induced cytotoxicity by TGF $\beta_1$  neutralizing antibody confirms the correlation between induction of active TGF $\beta_1$  and subsequent cell death [21]. The effect of a combination of mifepristone and 4-hydroxytamoxifen on cell growth inhibition, on the increase in DNA fragmentation, bcl<sub>2</sub> downregulation and induction of TGF $\beta_1$  protein was additive and significantly different ( $P < 0.05$ ) from the effect of monotherapy [21]. A translocation of protein kinase C (PKC) activity from the soluble to the particulate and/or nuclear fraction appeared to be also additive in cells treated with a combination of both 4-hydroxytamoxifen and mifepristone [21]. These results suggest that the mechanism of the additive antiproliferative activity of mifepristone and tamoxifen could be explained at least in part by an additive induction of apoptosis in both ER and PR positive MCF-7 breast cancer cells. A bcl<sub>2</sub> downregulation, the PKC transduction pathway, and TGF $\beta_1$  expression seem to be involved in this additive mechanism of action [21]. However, all clinical trials of concurrent combination endocrine therapy in breast cancer have so far not shown a confirmed evidence of increase either in the mean duration of remission or survival. Therefore, sequential endocrine therapy has been preferred because it keeps some effective therapy in reserve and may also delay the onset of tumor autonomy [22, 23]. Further-

more, there are no clinical data supporting that combinations of antiestrogens and antiprogestins should be better than monotherapy. Therefore, a lot of caution is needed in extrapolations of data from our experimental models to the clinical situation. Also, the precise molecular biochemical mechanism of the *in vivo* growth inhibitory effect of tamoxifen versus mifepristone in the MCF-7 tumor model needs to be clarified in future studies. These studies are currently going on in our laboratories using the breast cancer tissue harvested from the different nude mouse experiments described in this publication.

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ALOG(R) File 155: MEDLINE(R)  
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13041883 PMID: 11426648

Bcl - 2 family proteins as targets for anticancer drug design.

Huang Z

Department of Biochemistry, University of Illinois, Urbana 61801, USA.

Oncogene (England) Dec 27 2000, 19 (56) p6627-31, ISSN 0950-9232

--Print Journal Code: 8711562

Publishing Model Print

Document type: Journal Article; Review

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Bcl - 2 family proteins are key regulators of programmed cell death or apoptosis that is implicated in many human diseases, particularly cancer. In recent years, they have attracted intensive interest in both basic research to understand the fundamental principles of cell survival and cell death and drug discovery to develop a new class of anticancer agents. The Bcl - 2 family includes both anti- and pro-apoptotic proteins with opposing biological functions in either inhibiting or promoting cell death. High expression of anti-apoptotic members such as Bcl - 2 and Bcl - XL commonly found in human cancers contributes to neoplastic cell expansion and interferes with the therapeutic action of many chemotherapeutic drugs. The functional blockade of Bcl - 2 or Bcl - XL could either restore the apoptotic process in tumor cells or sensitize these tumors for chemo- and radiotherapies. This article reviews the recent progress in the design and discovery of small molecules that block the anti-apoptotic function of Bcl - 2 or Bcl - XL. These chemical inhibitors are effective modulators of apoptosis and promising leads for the further development of new anticancer agents.

Bcl - 2 family proteins as targets for anticancer drug design.

... 2000,

Bcl - 2 family proteins are key regulators of programmed cell death or apoptosis that is implicated in many...

... and cell death and drug discovery to develop a new class of anticancer agents. The Bcl - 2 family includes both anti- and pro-apoptotic proteins with opposing biological functions in either inhibiting or promoting cell death. High expression of anti-apoptotic members such as Bcl - 2 and Bcl - XL commonly found in human cancers contributes to neoplastic cell expansion and interferes with the therapeutic action of many chemotherapeutic drugs. The functional blockade of Bcl - 2 or Bcl - XL could either restore the apoptotic process in tumor cells or sensitize these tumors for chemo...

... in the design and discovery of small molecules that block the anti-apoptotic function of Bcl - 2 or Bcl - XL. These chemical inhibitors are effective modulators of apoptosis and promising leads for the further development...

Descriptors: \*Antineoplastic Agents--pharmacology--PD; \*Apoptosis--drug effects--DE; \*Drug Design; \*Proto-Oncogene Proteins c- bcl - 2 --antagonists and inhibitors--AI...; Peptides--chemistry--CH; Peptides --metabolism--ME; Peptides--pharmacology--PD; Protein Binding; Proto-Oncogene Proteins c- bcl - 2 --chemistry--CH; Proto-Oncogene Proteins c- bcl - 2 --metabolism--ME; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't...

Chemical Name: Antineoplastic Agents; BCL2L1 protein, human; Peptides; Proto-Oncogene Proteins c- **bcl** - 2 ; bcl-X Protein

16/3,K,AB/2 (Item 2 from file: 155)  
DIALOG(R) File 155: MEDLINE(R)  
(c) format only 2006 Dialog. All rts. reserv.

12914799 PMID: 11055382

Flavonoids inhibit cell growth and induce apoptosis in B16 melanoma 4A5 cells.

Iwashita K; Kobori M; Yamaki K; Tsushima T  
Institute of Applied Biochemistry, University of Tsukuba, Ibaraki, Japan.  
moldive@nfri.affrc.go.jp

Bioscience, biotechnology, and biochemistry (JAPAN) Sep 2000 , 64  
(9) p1813-20, ISSN 0916-8451--Print Journal Code: 9205717

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

We investigated the growth inhibitory activity of several flavonoids, including apigenin, luteolin, kaempferol, quercetin, butein, isoliquiritigenin, naringenin, genistein, and daizein against B16 mouse melanoma 4A5 cells. Isoliquiritigenin and butein, belonging to the chalcone group, markedly suppressed the growth of B16 melanoma cells and induced cell death. The other flavonoids tested showed little growth **inhibitory** activity and scarcely caused cell **death**. In cells treated with isoliquiritigenin or butein, condensation of nuclei and fragmentation of nuclear DNA, which are typical phenomena of apoptosis, were observed by Hoechst 33258 staining and by agarose gel electrophoresis of DNA. Flowcytometric analysis showed that isoliquiritigenin and butein increased the proportion of hypodiploid cells in the population of B16 melanoma cells. These results demonstrate that isoliquiritigenin and butein inhibit cell proliferation and induce apoptosis in B16 melanoma cells. Extracellular glucose decreased the proportion of hypodiploid cells that appeared as a result of isoliquiritigenin treatment. p53 was not detected in cells treated with either of these chalcones, however, protein of the **Bcl - 2 family** were detected. The level of expression of Bax in cells treated with either of these chalcones was markedly elevated and the level of **Bcl - XL** decreased slightly. Isoliquiritigenin did not affect **Bcl - 2** expression, but butein down-regulated **Bcl - 2** expression. From these results, it seems that the pathway by which the chalcones induce apoptosis may be independent of p53 and dependent on proteins of the **Bcl - 2 family**. It was supposed that isoliquiritigenin induces apoptosis in B16 cells by a mechanism involving inhibition of glucose transmembrane transport and promotion of Bax expression. On the other hand, it was suggested that butein induces apoptosis via down-regulation of **Bcl - 2** expression and promotion of Bax expression. This mechanism differs from the isoliquiritigenin induction pathway.

... 2000 ,  
... of B16 melanoma cells and induced cell death. The other flavonoids tested showed little growth **inhibitory** activity and scarcely caused cell **death**. In cells treated with isoliquiritigenin or butein, condensation of nuclei and fragmentation of nuclear DNA...

... was not detected in cells treated with either of these chalcones, however, protein of the **Bcl - 2 family** were detected. The level of

expression of Bax in cells treated with either of these chalcones was markedly elevated and the level of **Bcl - XL** decreased slightly. Isoliquiritigenin did not affect **Bcl - 2** expression, but butein down-regulated **Bcl - 2** expression. From these results, it seems that the pathway by which the chalcones induce apoptosis may be independent of p53 and dependent on proteins of the **Bcl - 2 family**. It was supposed that isoliquiritigenin induces apoptosis in B16 cells by a mechanism involving inhibition...

... On the other hand, it was suggested that butein induces apoptosis via down-regulation of **Bcl - 2** expression and promotion of Bax expression. This mechanism differs from the isoliquiritigenin induction pathway.

...; toxicity--TO; Luteolin; Melanoma, Experimental; Mice; Proto-Oncogene Proteins--analysis--AN; Proto-Oncogene Proteins c- **bcl - 2** --analysis--AN; Quercetin--toxicity--TO; Structure-Activity Relationship; Tumor Cells, Cultured; **bcl - 2** -Associated X Protein; bcl-X Protein

...Chemical Name: protein, mouse; Bcl211 protein, mouse; Flavanones; Flavonoids; Isoflavones;. Proto-Oncogene Proteins; Proto-Oncogene Proteins c- **bcl - 2** ; **bcl - 2** -Associated X Protein; bcl-X Protein; Quercetin; Genistein; naringenin; daidzein; butein; Luteolin; Apigenin; Chalcone; isoliquiritigenin

16/3,K,AB/3 (Item 3 from file: 155)  
DIALOG(R)File 155: MEDLINE(R)  
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12826561 PMID: 10949025

Aven, a novel inhibitor of caspase activation, binds Bcl - xL and Apaf-1.

Chau B N; Cheng E H; Kerr D A; Hardwick J M  
Department of Pharmacology and Molecular Sciences, Johns Hopkins University, Schools of Medicine and Public Health, Baltimore, Maryland 21205, USA.

Molecular cell (UNITED STATES) Jul 2000 , 6 (1) p31-40, ISSN 1097-2765--Print Journal Code: 9802571  
Contract/Grant No.: CA73581; CA; NCI; NS34175; NS; NINDS; NS37402; NS; NINDS

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Bcl-x(L), an antiapoptotic **Bcl - 2 family** member, is postulated to function at multiple stages in the cell death pathway. The possibility that Bcl-x(L) inhibits cell death at a late (postmitochondrial) step in the death pathway is supported by this report of a novel apoptosis inhibitor, Aven, which binds to both Bcl-x(L) and the caspase regulator, Apaf-1. Identified in a yeast two-hybrid screen, Aven is broadly expressed and is conserved in other mammalian species. Only those mutants of Bcl-x(L) that retain their antiapoptotic activity are capable of binding Aven. Aven interferes with the ability of Apaf-1 to self-associate, suggesting that Aven impairs Apaf-1-mediated activation of caspases. Consistent with this idea, Aven inhibited the proteolytic activation of caspases in a cell-free extract and suppressed apoptosis induced by Apaf-1 plus caspase-9. Thus, Aven represents a new class of cell death regulator.

Aven, a novel inhibitor of caspase activation, binds Bcl - xL and

**Apaf-1.**

... 2000 ,

Bcl-x(L), an antiapoptotic Bcl - 2 family member, is postulated to function at multiple stages in the cell death pathway. The possibility that Bcl-x(L) inhibits cell death at a late (postmitochondrial) step in the death pathway is supported by this report of...

...Descriptors: Proteins--metabolism--ME; \*Caspases--metabolism--ME; \*Membrane Proteins; \*Proteins--metabolism--ME; \*Proto-Oncogene Proteins c-bcl - 2 --metabolism--ME...; Sequence Data; Mutation; Protein Binding; Protein Structure, Quaternary; Proteins--chemistry--CH; Proto-Oncogene Proteins c-bcl - 2 --genetics--GE; RNA, Messenger--genetics--GE; RNA, Messenger--metabolism--ME; Research Support, Non-U.S...

...Chemical Name: BCL2L1 protein, human; Bcl2l1 protein

1/3,K,AB/7 (Item 7 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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15516130 PMID: 15922292

Unknotting the roles of Bcl - 2 and Bcl - xL in cell death.

Kim Ryungsa

International Radiation Information Center, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan.  
rkim@hiroshima-u.ac.jp

Biochemical and biophysical research communications (United States) Jul 29 2005, 333 (2) p336-43, ISSN 0006-291X--Print Journal Code: 0372516 Publishing Model Print

Document type: Journal Article; Review

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

The antiapoptotic Bcl - 2 family proteins Bcl - 2 and Bcl - xL play important roles in inhibiting mitochondria-dependent extrinsic and intrinsic cell death pathways. It seems that these two proteins have distinct functions for inhibiting extrinsic and intrinsic cell death pathways. The overexpression of Bcl - 2 is able to inhibit not only apoptotic cell death but also in part nonapoptotic cell death, which has the role of cell cycle arrest in the G1 phase, which may promote cellular senescence. The overexpression of Bcl - 2 may also have the ability to enhance cell death in the interaction of Bcl - xL with other factors. The overexpression of Bcl - xL enhances autophagic cell death when apoptotic cell death is inhibited in Bax(-/-)/Bak(-/-) double knockout cells. This review discusses the previously unexplained aspects of Bcl - 2 and Bcl - xL functions associated with cell death, for better understanding of their functions in the regulation.

Unknotting the roles of Bcl - 2 and Bcl - xL in cell death.

The antiapoptotic Bcl - 2 family proteins Bcl - 2 and Bcl - xL play important roles in inhibiting mitochondria-dependent extrinsic and intrinsic cell death pathways. It seems that these two proteins have distinct functions for inhibiting extrinsic and intrinsic cell death pathways. The overexpression of Bcl - 2 is able to inhibit not only apoptotic cell death but also in part nonapoptotic cell death, which has the role of cell cycle arrest in the G1 phase, which may promote cellular senescence. The overexpression of Bcl - 2 may also have the ability to enhance cell death in the interaction of Bcl - xL with other factors. The overexpression of Bcl - xL enhances autophagic cell death when apoptotic cell death is inhibited in Bax(-/-)/Bak(-/-) double knockout cells. This review discusses the previously unexplained aspects of Bcl - 2 and Bcl - xL functions associated with cell death, for better understanding of their functions in the regulation.

Descriptors: \*Apoptosis--physiology--PH; \*Mitochondria--metabolism--ME; \*Models, Biological; \*Proto-Oncogene Proteins c- bcl - 2 --metabolism--ME

Chemical Name: BCL2L1 protein, human; Proto-Oncogene Proteins c- bcl - 2 ; bcl-X Protein

11/3,K,AB/8 (Item 8 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

(c) format only 2006 Dialog. All rts. reserv.

15423641 PMID: 15625305

**Activation of PI3-kinase/PKB contributes to delay in neutrophil apoptosis after thermal injury.**

Hu Zhihong; Sayeed Mohammed M  
Dept. of Physiology, Loyola Univ. Medical Center, 2160 S. First Ave.,  
Maywood, IL 60153, USA. zhul@luc.edu

American journal of physiology. Cell physiology (United States) May 2005, 288 (5) pC1171-8, ISSN 0363-6143--Print Journal Code: 100901225 Contract/Grant No.: R01GM-52325; GM; NIGMS; R01GM-56865; GM; NIGMS

Publishing Model Print-Electronic  
Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Neutrophil apoptosis is delayed under trauma and/or sepsis injury conditions. The molecular mechanism for the delay in apoptosis has not been well defined. We investigated whether activation of phosphatidyl inositol 3-kinase (PI3-kinase)/PKB signaling pathway contributes to the delay in neutrophil apoptosis with thermal injury. Rats were subjected to burns (30% total body surface area, 98 degrees C for 10 s), and euthanized 24 h later. Blood neutrophils were isolated with the use of Ficoll gradient centrifugation and cultured for the indicated time periods. Apoptosis was determined using annexin V and PI labeling and flow cytometry. NF-kappaB activation was examined using gel mobility shift assay and confocal microscopy. Expression levels of inhibitory apoptosis proteins (IAPs), including cellular IAP1 (cIAP1), cIAP2, X-linked IAP (XIAP), and survivin, and Bcl - 2 family members such as Bcl - xl and Bad, were determined by Western blot analysis and/or RT-PCR, real-time PCR. The results showed that in culture, the decrease in apoptosis of neutrophils from thermally injured rats was prevented in the presence of PI3-kinase inhibitors wortmannin and LY-294002. There was upregulation of PKB and Bad phosphorylation and NF-kappaB activation in N-formyl-l-methionyl-l-leucyl-l-phenylalanine-stimulated neutrophils from thermally injured rats compared with the sham injured group. Increased Bad phosphorylation and NF-kappaB activation were also attenuated by wortmannin. Bcl - xl expression in neutrophils was upregulated with thermal injury and inhibited in the presence of wortmannin. However, the expression of IAP family members was neither affected by thermal injury nor inhibited by wortmannin. These data suggest that the delay in neutrophil apoptosis with thermal injury is partly caused by activation of PI3-kinase/PKB signaling and NF-kappaB, which appeared to be related to the increased Bcl - xl expression and phosphorylation of Bad, but not IAP expression.

... apoptosis proteins (IAPs), including cellular IAP1 (cIAP1), cIAP2, X-linked IAP (XIAP), and survivin, and Bcl - 2 family members such as Bcl - xl and Bad, were determined by Western blot analysis and/or RT-PCR, real-time PCR...

... sham injured group. Increased Bad phosphorylation and NF-kappaB activation were also attenuated by wortmannin. Bcl - xl expression in neutrophils was upregulated with thermal injury and inhibited in the presence of wortmannin...

... PI3-kinase/PKB signaling and NF-kappaB, which appeared to be related to the increased Bcl - xl expression and phosphorylation of Bad, but not IAP expression.

...; Oncogene Proteins--antagonists and inhibitors--AI; Proto-Oncogene Proteins c-akt; Proto-Oncogene Proteins c- bcl - 2 --metabolism--ME; Rats; Research Support, N.I.H., Extramural; Research Support, U.S. Gov't, P.H.S.; Signal Transduction--drug effects--DE; Signal Transduction--physiology--PH

; X-Linked Inhibitor of Apoptosis Protein; bcl-Associated Death Protein  
; bcl-X Protein

...Chemical Name: Enzyme Inhibitors; Inhibitor of Apoptosis Proteins; Morpholines; Proteins; Proto-Oncogene Proteins; Proto-Oncogene Proteins c-bcl - 2 ; X-Linked Inhibitor of Apoptosis Protein; bcl-Associated Death Protein; bcl-X Protein; 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one; wortmannin...

11/3,K,AB/9 (Item 9 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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15314216 PMID: 15683736

The survival of IL-6-dependent myeloma cells critically relies on their capability to transit the G1 to S phase interval of the cell cycle.

Cote Serge; Lemieux Real; Simard Carl  
Departement de Recherche et Developpement, Hema-Quebec, Sainte-Foy, QC, Canada. scote@hema-quebec.qc.ac

Cellular signalling (England) May 2005, 17 (5) p615-24, ISSN 0898-6568--Print Journal Code: 8904683

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed.

Interleukin-6 (IL-6) has an essential role in the initial progression of myeloma cell tumours. IL-6 triggers proliferation of these cells via the Ras-mitogen-activated protein kinase (MAPK) cascade and is thought to promote their survival via signal transducer and activator of transcription (STAT) pathway-dependent regulation of **Bcl - 2 family** antiapoptotic members. Using IL-6-dependent murine B9 hybridoma/plasmacytoma cells, we here report that exiting the cell cycle G1 phase is a crucial step contributing to maintain viability. We show that (1) drug-mediated reversible G1 arrest triggered apoptosis despite the presence of IL-6; (2) a short IL-6 pulse to G1-arrested cells was sufficient to induce S phase entry and prevent apoptosis; and (3) phorbol ester and related derivatives promoted S phase entry and survival of IL-6-starved cells without up-regulating **bcl - XL** expression. Furthermore, that the MAPK kinase (MEK) 1/2 **inhibitor**, U0126, blocked proliferation and induced **death** of B9 cells indicate that IL-6 may not exert its survival effect primarily through **bcl - XL** and emphasizes the key role of Ras-MAPK cascade elements in the regulation of myeloma growth/viability.

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... derivatives promoted S phase entry and survival of IL-6-starved cells without up-regulating **bcl - XL** expression. Furthermore, that the MAPK kinase (MEK) 1/2 **inhibitor**, U0126, blocked proliferation and induced **death** of B9 cells indicate that IL-6 may not exert its survival effect primarily through **bcl - XL** and emphasizes the key role of Ras-MAPK cascade elements in the regulation of myeloma...

...; G1 Phase--drug effects--DE; Hybridomas; Interleukin-6--pharmacology --PD; Mice; Proto-Oncogene Proteins c- **bcl - 2** --physiology--PH; S Phase;

Signal Transduction; Tetradecanoylphorbol Acetate--pharmacology--PD; bcl-X Protein

Chemical Name: Antigens, CD45; Bcl2l1 protein, mouse; Interleukin-6; Proto-Oncogene Proteins c- **bcl - 2**; bcl-X Protein; Tetradecanoylphorbol Acetate; Extracellular Signal-Regulated MAP Kinases

**poptin-induced cell death is modulated by Bcl - 2 family members and is Apaf-1 dependent.**

Burek M; Maddika S; Burek C J; Daniel P T; Schulze-Osthoff K; Los M  
Department of Immunology and Cell Biology, University of Munster,  
Munster, Germany.

Oncogene (England) Apr 6 2006, 25 (15) p2213-22, ISSN 0950-9232--  
Print Journal Code: 8711562

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Apoptin, a chicken anemia virus-derived protein, selectively induces apoptosis in transformed but not in normal cells, thus making it a promising candidate as a novel anticancer therapeutic. The mechanism of apoptin-induced apoptosis is largely unknown. Here, we report that contrary to previous assumptions, **Bcl - 2** and **Bcl - xL** inhibit apoptin-induced cell death in several tumor cell lines. In contrast, deficiency of Bax conferred resistance, whereas Bax expression sensitized cells to apoptin-induced death. Cell death induction by apoptin was associated with cytochrome c release from mitochondria as well as with caspase-3 and -7 activation. Benzylloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone, a broad spectrum caspase inhibitor, was highly protective against apoptin-induced cell death. Apoptosis induced by apoptin required Apaf-1, as immortalized Apaf-1-deficient fibroblasts as well as tumor cells devoid of Apaf-1 were strongly protected. Thus, our data indicate that apoptin-induced apoptosis is not only **Bcl - 2** - and caspase dependent, but also engages an Apaf-1 apotosome-mediated mitochondrial death pathway.

**Apoptin-induced cell death is modulated by Bcl - 2 family members and is Apaf-1 dependent.**

... of apoptin-induced apoptosis is largely unknown. Here, we report that contrary to previous assumptions, **Bcl - 2** and **Bcl - xL** inhibit apoptin-induced cell death in several tumor cell lines. In contrast, deficiency of Bax conferred resistance, whereas Bax expression...

... 1 were strongly protected. Thus, our data indicate that apoptin-induced apoptosis is not only **Bcl - 2** - and caspase dependent, but also engages an Apaf-1 apotosome-mediated mitochondrial death pathway.

...Descriptors: PD; \*Intracellular Signaling Peptides and Proteins--metabolism--ME; \*Proteins--metabolism--ME; \*Proto-Oncogene Proteins c-  
**bcl - 2**--metabolism--ME; \*bcl-X Protein--metabolism--ME...; Neoplasms--pathology--PA; Proteins--antagonists and inhibitors--AI; Proteins--genetics--GE; Proto-Oncogene Proteins c-  
**bcl - 2**--genetics--GE; RNA, Small Interfering--pharmacology--PD; Research Support, Non-U.S. Gov't; Tumor...

...Chemical Name: Capsid Proteins; Cysteine Proteinase Inhibitors; Intracellular Signaling Peptides and Proteins; Proteins; Proto-Oncogene Proteins c-  
**bcl - 2**; RNA, Small Interfering; VP3 protein, Chicken anemia virus; apoptotic protease-activating factor 1; bcl-X...

11/3,K,AB/3 (Item 3 from file: 155)  
DIALOG(R)File 155: MEDLINE(R)  
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19787271 PMID: 16332193

Characterization of the effects of Bcl - 2 and Bcl - xL deletion

**mutant expression in cell lines used for antibody production.**

Peterson Norman C; Servinsky Matthew  
Department of Comparative Medicine, Johns Hopkins University, Baltimore,  
Maryland, USA. petersonn@medimmune.com

Hybridoma (2005) (United States) Dec 2005, 24 (6) p275-82, ISSN  
1554-0014--Print Journal Code: 101241539

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Strategies to maximize monoclonal antibody (MAb) yields by in vitro production methods entail that hybridoma cells be maintained at high density. Approaches to increase culture density and antibody yields from hybridomas by inhibiting apoptosis through over-expression of exogenous

Bcl - 2 family genes have pro

FI Publication Control No: 2006-0083738 IFI Chemical Acc No: 2006-0018352  
Document Type: C

**TREATMENT OF CANCER BY THE USE OF ANTI FAS ANTIBODY**

Inventors: Johnston Patrick Gerard (GB); Longley Daniel (GB)

Assignee: FUSION ANTIBODIES Ltd GB

Attorney, Agent or Firm: DRINKER BIDDLE & REATH;ATTN: INTELLECTUAL PROPERTY GROUP, ONE LOGAN SQUARE, 18TH AND CHERRY STREETS, PHILADELPHIA, PA, 19103-6996, US

Publication (No,Kind,Date), Applic (No,Date):

US 20060083738 A1 20060420 US 2003514604 20030516

Internat. Convention Pub(No,Date),Applic(No,Date):

WO 2003GB210 20030516

Section 371: 20051202

Section 102(e):20051202

Priority Applic(No,Date): GB 2002113777 20020517; GB 2002148856  
20020627

**Abstract:** The present invention provides a method of killing cancer cells and method of treatment of cancer comprising administration of a therapeutically effective amount of a) a specific binding member which binds to a cell **death receptor** or a nucleic acid encoding said binding member and (b) a chemotherapeutic agent. The binding member preferably binds to a **Fas receptor**. Also described are medicaments for use in treating cancer.

```
? ds

Set      Items      Description
S1       25        DEATH(W) RECEPTOR??
S2       614       FAS
S3       11        S1 AND S2
? s s1 not s3
      25  S1
      11  S3
      S4  14  S1 NOT S3
? t s4/3,k,ab/1-10
```

**4/3,K,AB/1**

ialog Acc No: 10521444 IFI Acc No: 2004-0028658  
IFI Publication Control No: 2004-0028658 IFI Chemical Acc No: 2004-0007890  
Document Type: C

**METHODS OF ORGAN REGENERATION; INDUCE CELL DIFFERENTIATION; PROCESS CONTROL**

Inventors: Faustman Denise (US)

Assignee: Unassigned Or Assigned To Individual

Assignee Code: 68000

Probable Assignee: General Hospital Corp The

Attorney, Agent or Firm: CLARK & ELBING LLP, 101 FEDERAL STREET, BOSTON, MA  
, 02110, US

Publication (No,Kind,Date), Applic (No,Date):

US 20040028658 A1 20040212 US 2003358664 20030205

Priority Applic(No,Date): US 2003358664 20030205

Provisional Applic(No,Date): US 60-392687 20020627

**Abstract:** The invention features methods for increasing or maintaining the number of functional cells of a predetermined type, for example, insulin producing cells of the pancreas, blood cells, spleen cells, brain cells, heart cells, vascular tissue cells, cells of the bile duct, or skin cells, in a mammal (e.g., a human patient) that has injured or damaged cells of the predetermined type.

**Non-exemplary Claims:**

...said cells that differentiate into cells of said predetermined type in vivo fail to express **Fas** or **FasL**...

...The method of claim 1, 9, or 15, wherein said composition binds or activates a **death receptor**.

...

... 9, 11-15, and 21 further comprising administering